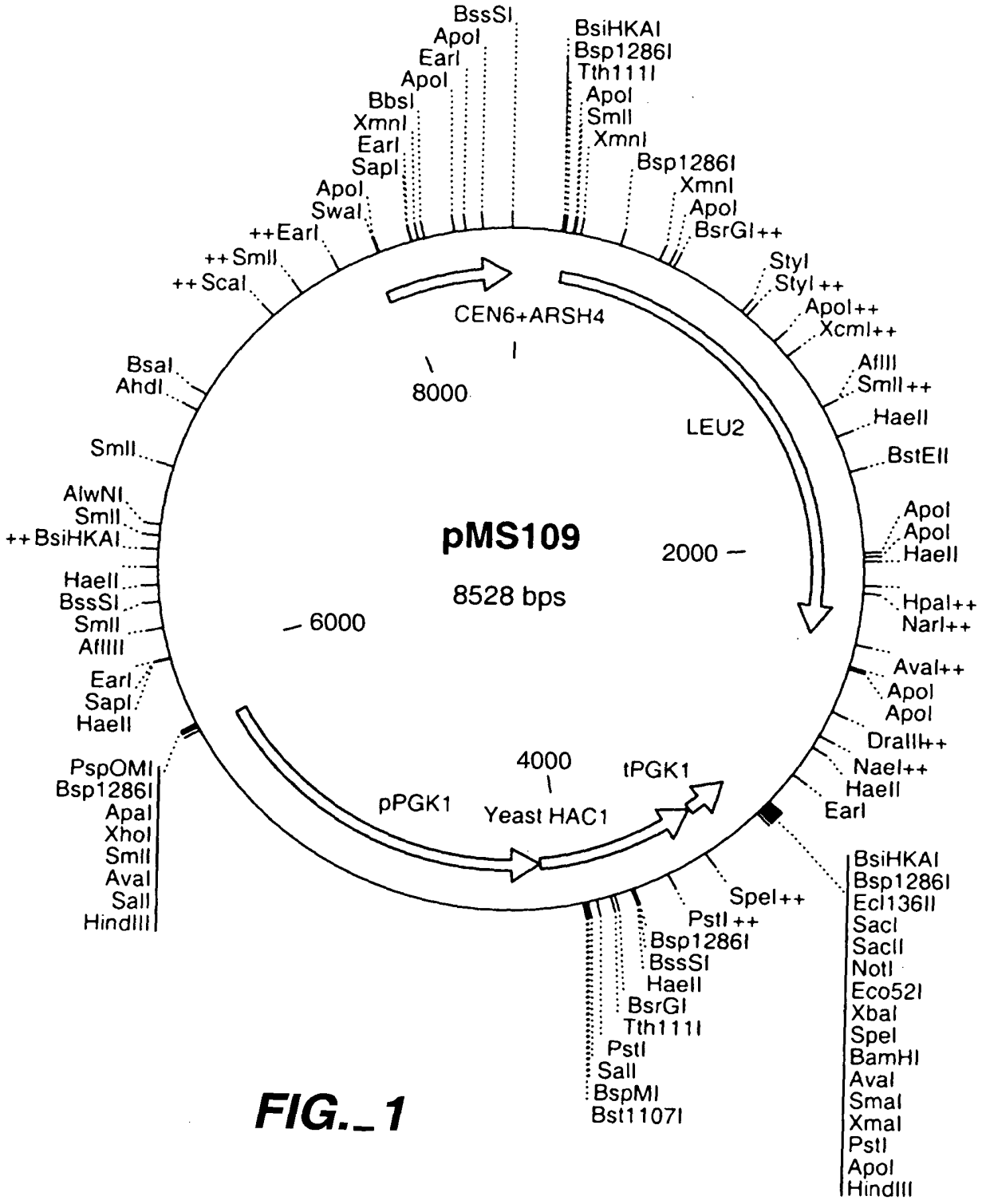

pMS109
8528 bps
CEN6+ARSH4
LEU2
pPGK1
Yeast HAC1
tPGK1
8000
2000
4000
6000
BssSI
ApoI
EarI
ApoI
BbsI
XmnI
EarI
SapI
ApoI
SwaI
++EarI
++SmlI
++ScaI
BsaI
AhdI
SmlI
AlwNI
SmlI
++BsiHKAI
HaeII
BssSI
SmlI
AflIII
EarI
SapI
HaeII
PspOMI
Bsp1286I
ApaI
XhoI
SmlI
AvaI
SalI
HindIII
BsiHKAI
Bsp1286I
Tth111I
ApoI
SmlI
XmnI
Bsp1286I
XmnI
ApoI
BsrGI++
StyI
StyI++
ApoI++
XcmI++
AflII
SmlI++
HaeII
BstEII
ApoI
ApoI
HaeII
HpaI++
NarI++
AvaI++
ApoI
ApoI
DraIII++
NaeI++
HaeII
EarI
SpeI++
PstI++
Bsp1286I
BssSI
HaeII
BsrGI
Tth111I
PstI
SalI
BspMI
Bst1107I
BsiHKAI
Bsp1286I
Ecl136II
SacI
SacII
NotI
Eco52I
XbaI
SpeI
BamHI
AvaI
SmaI
XmaI
PstI
ApoI
HindIII

FIG._1

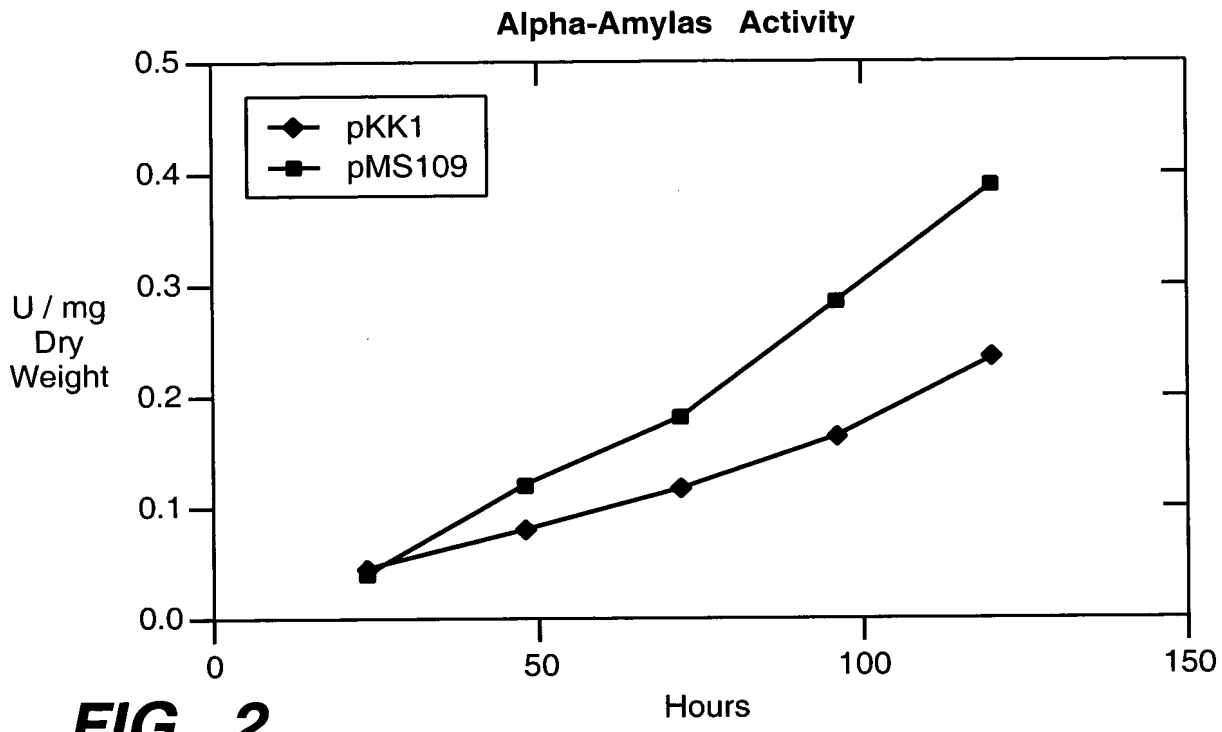

Alpha-Amylas Activity
pKK1
pMS109
U / mg Dry Weight
0.5
0.4
0.3
0.2
0.1
0.0
0
50
100
150
Hours

FIG._2

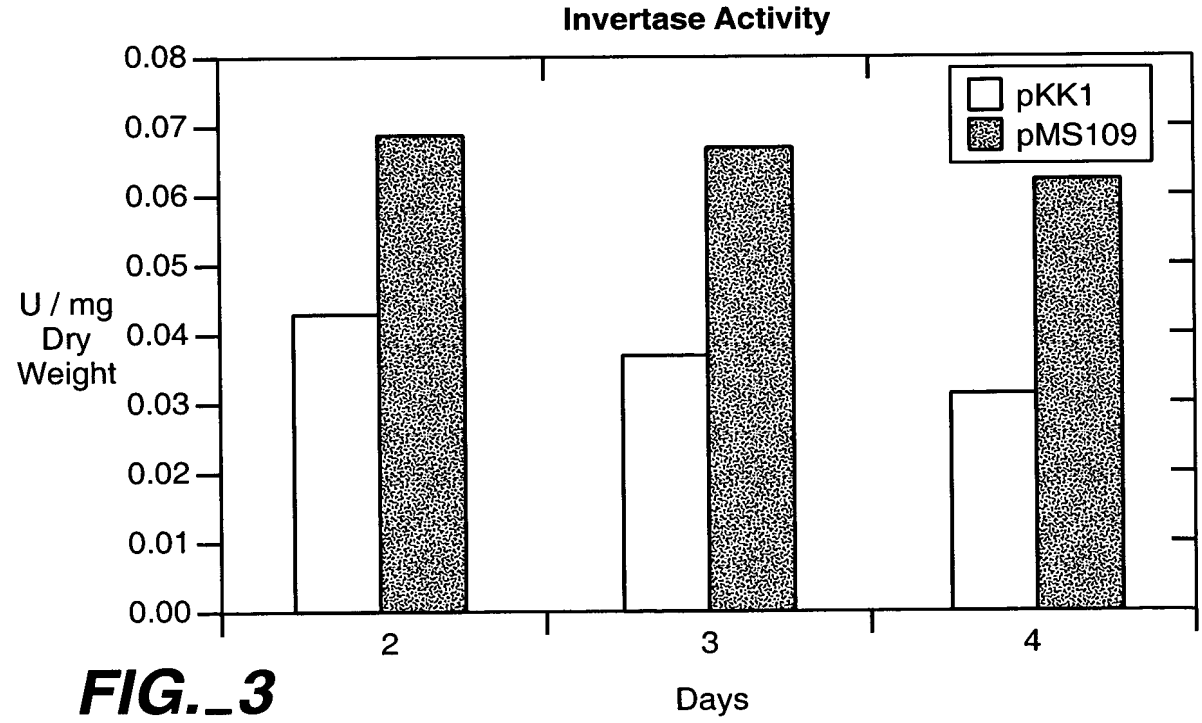

Invertase Activity
pKK1
pMS109
U / mg Dry Weight
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
2
3
4
Days

FIG._3

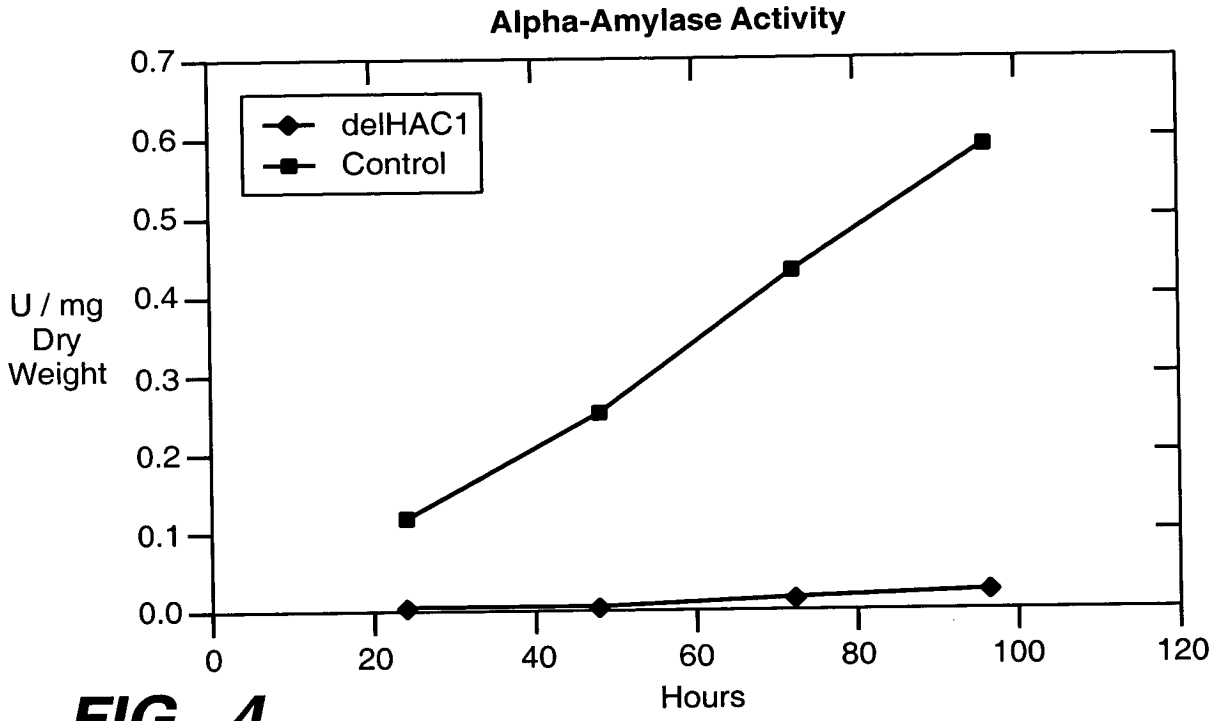

Alpha-Amylase Activity
delHAC1
Control
U / mg Dry Weight
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0
20
40
60
80
100
120
Hours

FIG._4

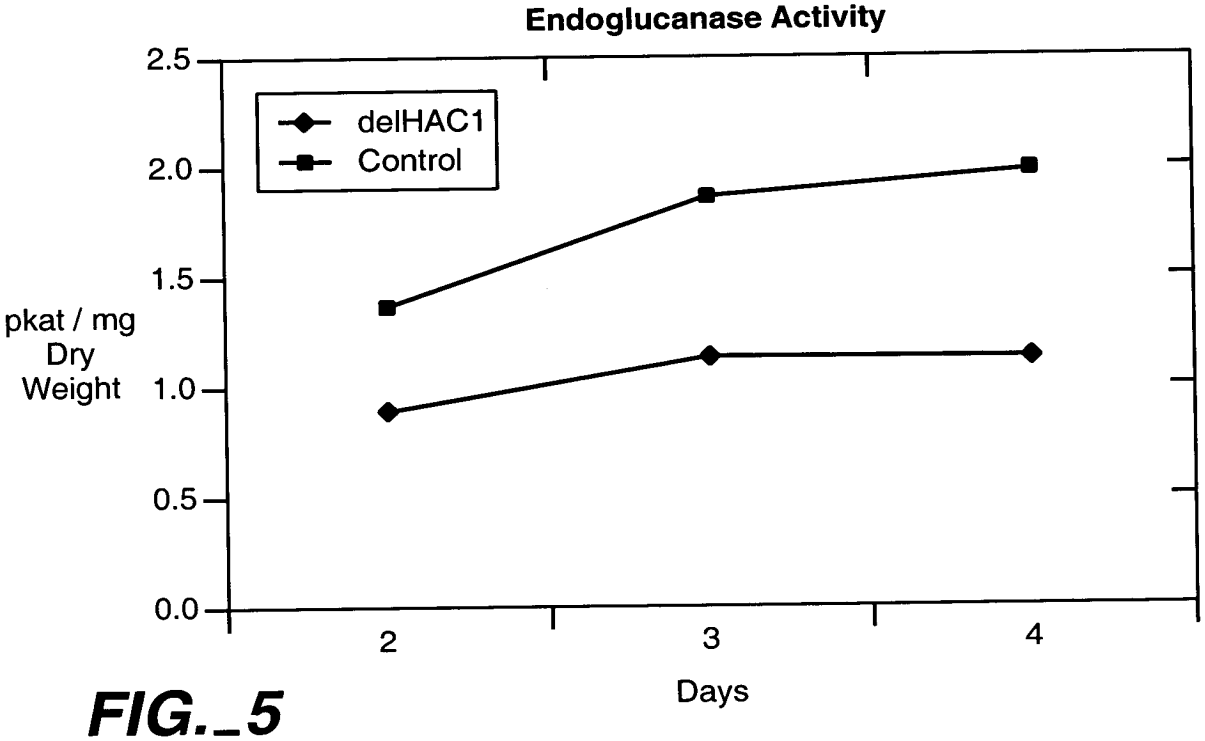
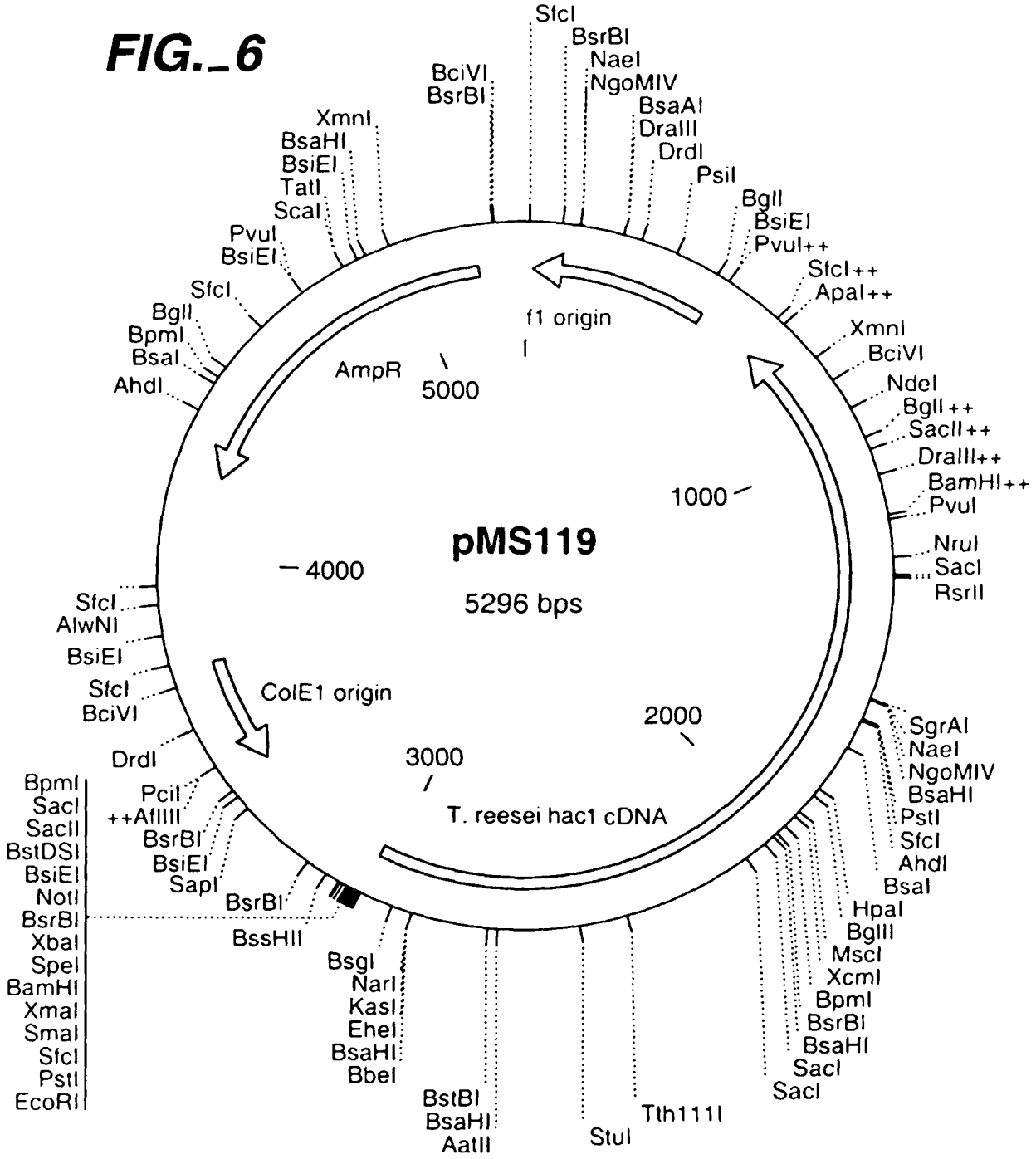

Endoglucanase Activity
delHAC1
Control
pkat / mg Dry Weight
0.0
0.5
1.0
1.5
2.0
2.5
2
3
4
Days

FIG._5

FIG._6


pMS119
5296 bps
f1 origin
AmpR
5000
1000
2000
3000
4000
ColE1 origin
T. reesei hac1 cDNA
SfcI
BsrBI
NaeI
NgoMIV
BsaAI
DraIII
DrdI
PsiI
BglI
BsiEI
PvuI++
SfcI++
ApaI++
XmnI
BciVI
NdeI
BglI++
SacII++
DraIII++
BamHI++
PvuI
NruI
SacI
RsrII
SgrAI
NaeI
NgoMIV
BsaHI
PstI
SfcI
AhdI
BsaI
HpaI
BglII
MscI
XcmI
BpmI
BsrBI
BsaHI
SacI
SacI
Tth111I
StuI
BstBI
BsaHI
AatII
BsgI
NarI
KasI
EheI
BsaHI
BbeI
BsrBI
BssHII
BsrBI
SapI
BsiEI
BsrBI
++AflIII
PciI
DrdI
SfcI
BciVI
SfcI
BsiEI
AlwNI
SfcI
BpmI
SacI
SacII
BstDSI
BsiEI
NotI
BsrBI
XbaI
SpeI
BamHI
XmaI
SmaI
SfcI
PstI
EcoRI
AhdI
BsaI
BpmI
AflIII
BglI
SfcI
PvuI
BsiEI
TatI
ScaI
BsaHI
BsiEI
XmnI
BciVI
BsrBI

```
GCAGAGGCCACTCTGTCCTCTTCTGCCTGACTCATCACTCCTCGACAGCATCACCAAGGGGAACGCACTGCACTTGGACACAGCCACGCC   90

GCTTCCCACTGACTCATTTGGGACTGGCGCCGTTGCCTGTCATGACTGTTCGCATCGTCGTCATCAACCATCGACTGACACGCTTCGCTT  180

TGATTTGATTGCTTCTCTCTCCACTCTCTCTCTTCCTGTCTCTCTACTACTACTACTACTCTCTCTTCTGCATCTCCACCGGCCTGTGAC  270

CGAAAAAACCAACTCCGTCTCCTTTCGAAGAAGAAACAGTTGGTCCGACGTCACAAGCACATTCACAAAAATCAAACAACATATCCCCAT  360

CTTTCATATACACCACACGCTTATGCAGTGAGAGAGCACGAGAGAAGCATCGTCATAATCAACACATCAGTCAAAGCGAACTGCGCTCGG  450

CAACACGACACGGCAGGCAACATGGCGTTCCAGCAGTCGTCTCCCCTCGTCAAGTTTGAGGCCTCTCCCGCCGAATCCTTCCTCTCCGCC  540
                      M  A  F  Q  Q  S  S  P  L  V  K  F  E  A  S  P  A  E  S  F  L  S  A

CCCGGCGACAACTTCACATCCCTCTTCGCCGACTCAACACCCTCAACACTTAACCCTCGGGACATGATGACCCCTGACAGCGTCGCCGAC  630
 P  G  D  N  F  T  S  L  F  A  D  S  T  P  S  T  L  N  P  R  D  M  M  T  P  D  S  V  A  D

ATCGACTCTCGCCTGTCCGTCATCCCCGAATCACAGGACGCGGAAGATGACGAATCACACTCCACATCCGCTACCGCACCCTCTACCTCA  720
 I  D  S  R  L  S  V  I  P  E  S  Q  D  A  E  D  D  E  S  H  S  T  S  A  T  A  P  S  T  S

GAAAAGAAGCCCGTCAAGAAGAGGAAATCATGGGGCCAGGTTCTTCCTGAGCCCAAGACCAACCTCCCTCCTCGgtatgtcactgcaaca  810
 E  K  K  P  V  K  K  R  K  S  W  G  Q  V  L  P  E  P  K  T  N  L  P  P  R

cggctcacttgatacaacttgcatcctaaccaaacgttactgtagAAAACGTGCAAAGACGGAAGATGAAAAGGAGCAGCGCCGCGTCGA  900
                                                K  R  A  K  T  E  D  E  K  E  Q  R  R  V  E

GCGTGTTCTCCGCAACCGCCGCGCCGCGCAGTCCTCGCGCGAGCGCAAGAGGCTCGAGGTCGAGGCTCTCGAGAAGCGCAACAAGGAGCT  990
  R  V  L  R  N  R  R  A  A  Q  S  S  R  E  R  K  R  L  E  V  E  A  L  E  K  R  N  K  E  L

CGAGACGCTCCTCATCAACGTCCAGAAGACCAACCTGATCCTCGTCGAGGAGCTCAACCGCTTCCGACGCAGCTCAGGCGTCGTCACCCG 1080
  E  T  L  L  I  N  V  Q  K  T  N  L  I  L  V  E  E  L  N  R  F  R  R  S  S  G  V  V  T  R
```

FIG._7A

```
CTCGTCCTCCCCCCTCGACTCTCTCCAGGACAGCATCACTCTCTCCCAGCAACTCTTTGGCTCGCGGGATGGCCAAACCATGTCCAACCC 1170
  S  S  S  P  L  D  S  L  Q  D  S  I  T  L  S  Q  Q  L  F  G  S  R  D  G  Q  T  M  S  N  P

CGAGCAGTCCTTGATGGACCAGATCATGAGATCTGCCGCTAACCCTACCGTTAACCCGGCCTCTCTTTCCCCCTCCCTCCCCCCCATCTC 1260
  E  Q  S  L  M  D  Q  I  M  R  S  A  A  N  P  T  V  N  P  A  S  L  S  P  S  L  P  P  I  S

GGACAAGGAGTTCCAGACCAAGGAGGAGGACGAGGAACAGGCCGACGAAGATGAAGAGATGGAGCAGACATGGCACGAGACCAAAGAAGC 1350
  D  K  E  F  Q  T  K  E  E  D  E  E  Q  A  D  E  D  E  E  M  E  Q  T  W  H  E  T  K  E  A

CGCCGCCGCCAAGGAGAAGAACAGCAAGCAGTCCCGCGTCTCCACTGATTCGACACAACGTCCTGCagagatgttgtgcgacccgcAGTG 1440
  A  A  A  K  E  K  N  S  K  Q  S  R  V  S  T  D  S  T  Q  R  P  A                       V

TCAATCGGTGGAGATGCCGCTGTCCCTGTCTTCTCAGACGACGCCGGCGCAAACTGCCTTGGCCTGGACCCTGTTCATCAGGATGATGGT 1530
  S  I  G  G  D  A  A  V  P  V  F  S  D  D  A  G  A  N  C  L  G  L  D  P  V  H  Q  D  D  G

CCTTTCAGCATCGGCCATTCTTTCGGCCTGTCAGCGGCCCTTGATGCAGATCGCTATCTCCTCGAAAGCCAACTTCTCGCTTCGCCCAAC 1620
  P  F  S  I  G  H  S  F  G  L  S  A  A  L  D  A  D  R  Y  L  L  E  S  Q  L  L  A  S  P  N

GCCTCAACTGTTGACGACGATTATCTGGCTGGTGACTCTGCCGCCTGCTTCACGAATCCTCTCCCCTCCGACTACGACTTCGACATCAAC 1710
  A  S  T  V  D  D  D  Y  L  A  G  D  S  A  A  C  F  T  N  P  L  P  S  D  Y  D  F  D  I  N

GACTTCCTCACAGACGACGCAAACCACGCCGCCTATGACATTGTGGCAGCGAGCAACTATGCCGCTGCGGACCGCGAGCTCGACCTCGAG 1800
  D  F  L  T  D  D  A  N  H  A  A  Y  D  I  V  A  A  S  N  Y  A  A  A  D  R  E  L  D  L  E

ATCCACGACCCTGAGAATCAGATCCCTTCGCGACATTCTATCCAGCAGCCCCAGTCTGGCGCGTCCTCTCATGGATGCGACGATGGCGGC 1890
  I  H  D  P  E  N  Q  I  P  S  R  H  S  I  Q  Q  P  Q  S  G  A  S  S  H  G  C  D  D  G  G

ATTGCGGTTGGTGTCTGAGGGACGCGACGATCGGGGCGGGATCCCGGCCTCCGAGTCTTGTGCGACGCGCGGCGACTGCGAGCTGGAACG 1980
  I  A  V  G  V
```

FIG._7B

GTGCCTACGCAGCGTGACCTTGCCGTCTCGAGAAGTCCTCATCACCCTGTGGTGGGCCGTGAAGGTGGAGGAGAGGAGGATTCGCCTGAG 2070

GCAGCACAAGAAGCAGGCCGCGGCTCTCGACCCCGAGAAGCGCGCCTCCTTGGCAGACAAGAAGAACCGACAACAACAACAACAACAACA 2160

CCAGTATCAGATTCCTTCGTTTTCAAAATAGTTAGCATATGTGGTTTTTTAATGGGCAATGGGGCGGATGGCAACACGGTAGAGGCAACA 2250

AGGGTTGACTACACCTCCCAAAGGGATACGGCGCACAGCGAGGTTAATGACAAGGCTAAGATGGGCCTTTTTTTTTTTATGATATGAGAAC 2340

CTCTTCATCTCCCTTTACACTTCTCTCTAGATGGTAGTGATGATATACTGTACCAAAATACAACGTCTACCTAGTGCT 2418

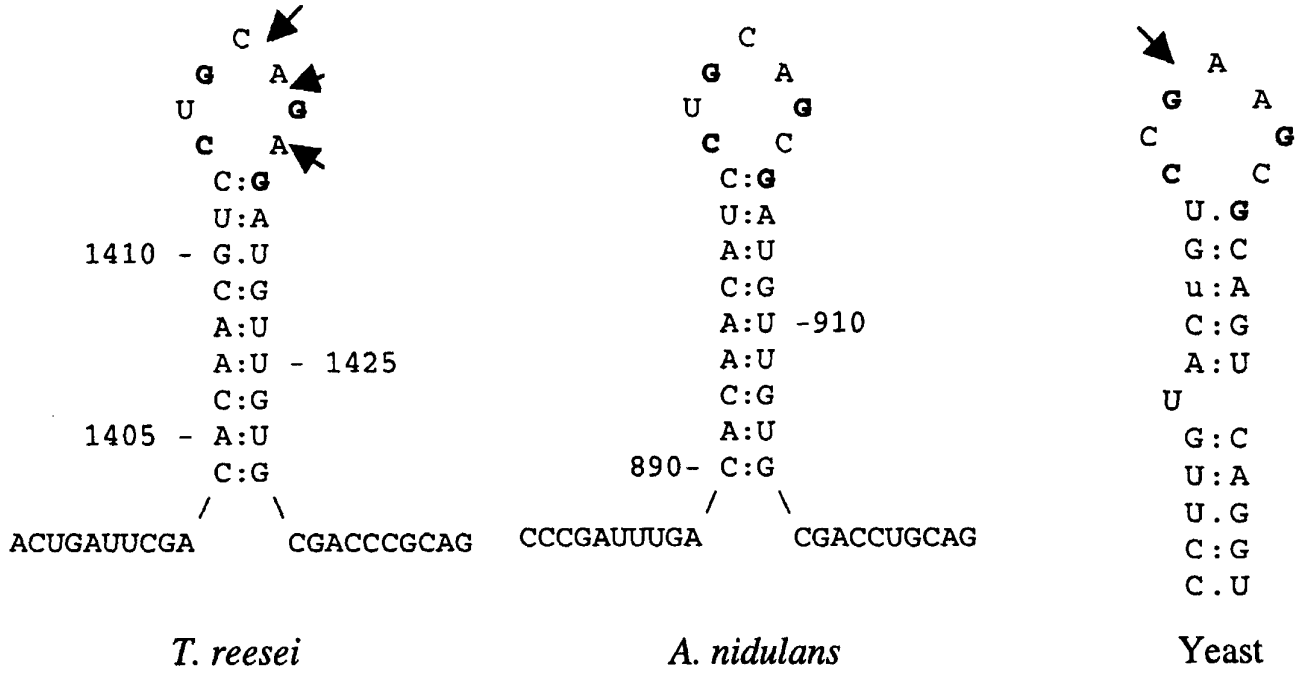
FIG._7C

```
GCCATCCTTGGTGACTGAGCCCCAACACTTTCACTGGTCGGGATAGTAGCCTCTGGCTTCGATTCGCTATGACACCGTGGCCTCTGTCCT    90

AAGTGACTCAGGCAAGGCAATCCCAGTTCCAACTCCCAACTTCGCAACCTCATCAACCACCTGCTTCCGTCTAGTTGCAGTTATCAGACT   180

TGAGTTGTATGAAATCAGCAGACCGGTTTTCGCCAGTGAAAATGGAGGACGCTTTCGCAAACTCTTTGCCTACTACCCCGTCATTGGAGG   270
        M  K  S  A  D  R  F  S  P  V  K  M  E  D  A  F  A  N  S  L  P  T  T  P  S  L  E

TTCCTGTGCTCACTGTCTCCCCGGCTGACACATCTCTTCGGACGAAGAATGTGGTGGCTCAGACAAAGCCTGAGGAGAAGAAGCCAGCGA   360
V  P  V  L  T  V  S  P  A  D  T  S  L  R  T  K  N  V  V  A  Q  T  K  P  E  E  K  K  P  A

AGAAAAGAAAGTCCTGGGGCCAGGAATTACCAGTTCCCAAGACAAACTTACCTCCAAGgtgtgtgatacctcaagagtcaactccttact   450
K  K  R  K  S  W  G  Q  E  L  P  V  P  K  T  N  L  P  P  R

cctgctaataactaccacagAAAACGCGCTAAGACAGAAGATGAGAAAGAGCAGCGCCGGATTGAGCGAGTTCTTCGCAACCGCGCAGCC   540
                     K  R  A  K  T  E  D  E  K  E  Q  R  R  I  E  R  V  L  R  N  R  A  A

GCACAAACCTCTCGCGAGCGCAAGAGACTTGAAATGGAGAAGTTAGAAAGCGAGAAGATTGATATGGAACAACAAAACCAGTTCCTTCTT   630
 A  Q  T  S  R  E  R  K  R  L  E  M  E  K  L  E  S  E  K  I  D  M  E  Q  Q  N  Q  F  L  L

CAGCGTCTCGCCCAGATGGAGGCTGAGAACAACCGTTTAAGTCAGCAAGTTGCTCAGCTATCCGCGGAGGTTCGGGGATCCCGCCACAGC   720
 Q  R  L  A  Q  M  E  A  E  N  N  R  L  S  Q  Q  V  A  Q  L  S  A  E  V  R  G  S  R  H  S

ACTCCAACTTCCAGTTCCCCCGCGTCAGTTTCGCCAACTCTCACACCGACTCTTTTTAAGCAGGAAGGGGATGAGGTTCCTCTGGACCGC   810
 T  P  T  S  S  S  P  A  S  V  S  P  T  L  T  P  T  L  F  K  Q  E  G  D  E  V  P  L  D  R

ATCCCTTTTCCAACTCCCTCCGTGACCGACTACTCCCCAACTCTTAAGCCTTCATCTCTGGCTGAGTCCCCCGATTTGACACAACATCCT   900
 I  P  F  P  T  P  S  V  T  D  Y  S  P  T  L  K  P  S  S  L  A  E  S  P  D  L  T  Q  H  P

GCagcgatgttgtgcgacctgcAGTGTCAGTCGGCGGGCTCGAAGGAGATGAAAGTGCCCTCACGCTTTTCGACCTCGGAGCCAGCATTA   990
 A                      V  S  V  G  G  L  E  G  D  E  S  A  L  T  L  F  D  L  G  A  S  I
```

FIG._8A

```
AGCATGAGCCTACACATGACCTTACAGCTCCTCTTTCTGACGATGACTTCCGCCGCCTATTCAACGGTGATTCATCCCTTGAGTCAGATT 1080
K  H  E  P  T  H  D  L  T  A  P  L  S  D  D  D  F  R  R  L  F  N  G  D  S  S  L  E  S  D

CTTCACTCCTTGAAGACGGGTTCGCCTTTGACGTTCTCGACTCAGGAGATTTATCAGCATTTCCATTTGATTCTATGGTTGATTTTGACA 1170
S  S  L  L  E  D  G  F  A  F  D  V  L  D  S  G  D  L  S  A  F  P  F  D  S  M  V  D  F  D

CCGAGCCTGTCACCCTCGAAGATCTCGAGCAAACCAACGGCCTTTCGGATTCAGCTTCTTGCAAGGCTGCTAGCTTGCAACCCAGCCATG 1260
T  E  P  V  T  L  E  D  L  E  Q  T  N  G  L  S  D  S  A  S  C  K  A  A  S  L  Q  P  S  H

GCGCGTCCACTTCGCGATGCGACGGGCAGGGCATTGCAGCTGGCAGTGCGTGAGAGGTTTTCGACGGAAGACCGTCTGGTTCCCGATGTT 1350
G  A  S  T  S  R  C  D  G  Q  G  I  A  A  G  S  A

GTAGAGGGTCGATGGAGCTGGGAATCCTTGTTAACGCTAGCGTCGGCGATAAATCTTCTTGAGAAACCGGAGCGACGAAGAAGAACCTTG 1440

AGGGGTCTTGATTCGTTAAAGCGGGGTCGGCGTATTGATTCGGGGAAGCGGTACAGGGTCATACGGAGTTCACGGAGTTCAACTAGCCCA 1350

AGAGAGGCGTTGACGTCTCGGAGAAAGGGCTTATGATAATTTGTATATTAGCGTGTCCACTATTCAATGTAAGAGCGAGCAATTG 1615
```

FIG._8B



T. reesei CCACTGATTCGACACAACGTCCTGCagagatgttgtgcgacccgcAGTGTCAATCGGTGG 1451
A. nidulans CCCCCGATTTGACACAACATCCTGCagcgatgttgtgcgacctgcAGTGTCAGTCGGCGG 937
** * **** ******** ******** ********** ********* **** **

FIG._9

```
T. reesei    MAFQQSSPLVKFEASPAESFLSAPGDNFTSLFADSTPSTLNPRDMMTPDS  50
A. nidulans  MKSADRFSPVKMEDA----------------FANSLPTTPSLEVPVLTVS  34
             *        ** * .                ** * *.*      .   *

T. reesei    VADIDSRLSVIPESQDAEDDESHSTSATAPSTSEKKPVKKRKSWGQVLPE 100
A. nidulans  PADTSLRTKNVVAQTKPE---------------EKKPAKKRKSWGQELPV  69
              **   *   .      *                **** ******** **

T. reesei    PKTNLPPRKRAKTEDEKEQRRVERVLRNRRAAQSSRERKRLEVEALEKRN 150
A. nidulans  PKTNLPPRKRAKTEDEKEQRRIERVLRNRAAAQTSRERKRLEMEKLESEK 119
Yeast        *KSTLPPRKRAKTKEEKEQRRIERILRNRRAAHQSREKKRLHLQYLERKC  71
              *. ********* .******.**.**** **  ***.*** .. **

T. reesei    KELETLLINVQKTNLILVEELNRFRRSSGVVTRSSSPLDSLQDSITLSQQ 200
A. nidulans  IDMEQQN---QFLLQRLAQMEAENNRLSQQVAQLSAEVRGSRHSTPTSSS 166
Yeast        SLLENLLNSVNLEK--LADHE    *  * *.  *. .     *   *
               .*      .     * .

T. reesei    LFGSRDGQTMSNPEQSLMDQIMRSAANPTVNPASLSPSLPPISDKEFQTK 250
A. nidulans  PASVSPTLTPTLFKQEGDEVPLDRIPFFPTPSVTDYSPTLKPSSLAE---- 212
                     * .   *   .  .     **   .  **.* * *  *

T. reesei    EEDEEQADEDEEMEQTWHETKEAAAAKEKNSKQSRVSTDSTQRPAVSIGG 300
A. nidulans  ------------------------------------SPDLTQHPAVSVGG 226
                                                 * * ** ****.**

T. reesei    DAAVPVFSDDAGANCLGLDPVHQDDGPFSIGHSFGLSAALDADRYLLESQ 350
A. nidulans  LEGDESALTL---FDLGASIKHEPTHDLTAPLSDDDFRRLFNGDSSLESD 273
                          **    *      .   *      *      ***

T. reesei    LLASPNASTVDDDYLAGDSAACFTNPLPSDYDFDINDFLTDDANHAAYDI 400
A. nidulans  SSLLEDGFAFDV----LDSGDLSAFPFDSMVDFDTEPVTLEDLEQTNGLS 319
                     . *      **    . *  *  ***      .*   .

T. reesei    VAASNYAAADRELDLEIHDPENQIPSRHSIQQPQSGASSHGCDDGGIAVGV 451
A. nidulans  DSASCKAASL---------------------QPSHGASTSRCDGQGIAAGSA 350
              .**  **.                     **  ***.  **  *** *
```

FIG._10

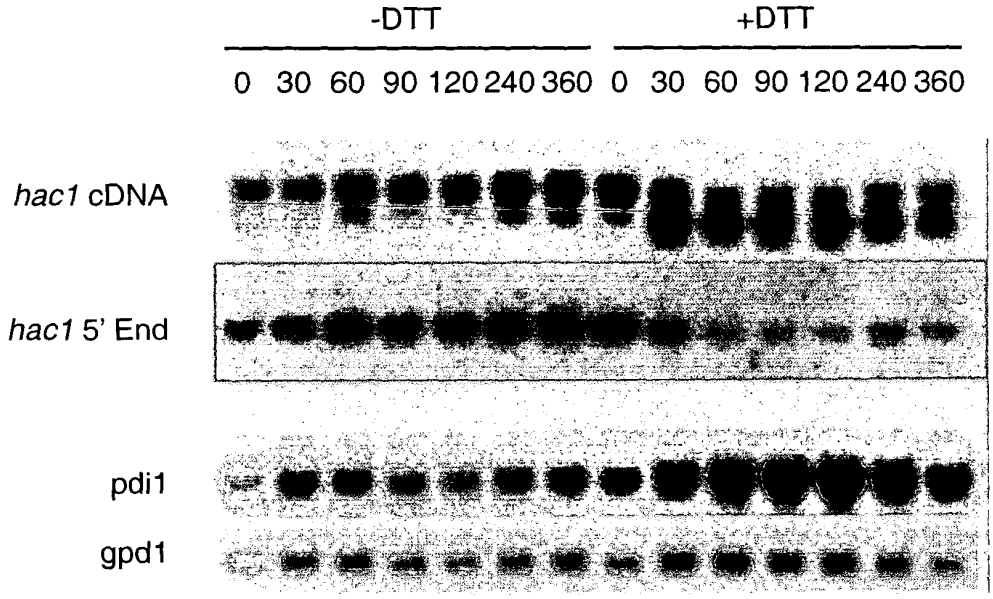

-DTT
+DTT
0 30 60 90 120 240 360 0 30 60 90 120 240 360
hac1 cDNA
hac1 5' End
pdi1
gpd1

FIG._11

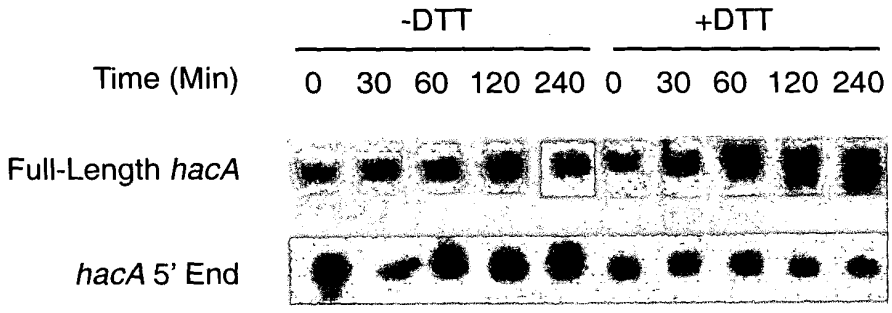

-DTT
+DTT
Time (Min)
0 30 60 120 240 0 30 60 120 240
Full-Length *hacA*
hacA 5' End

FIG._12

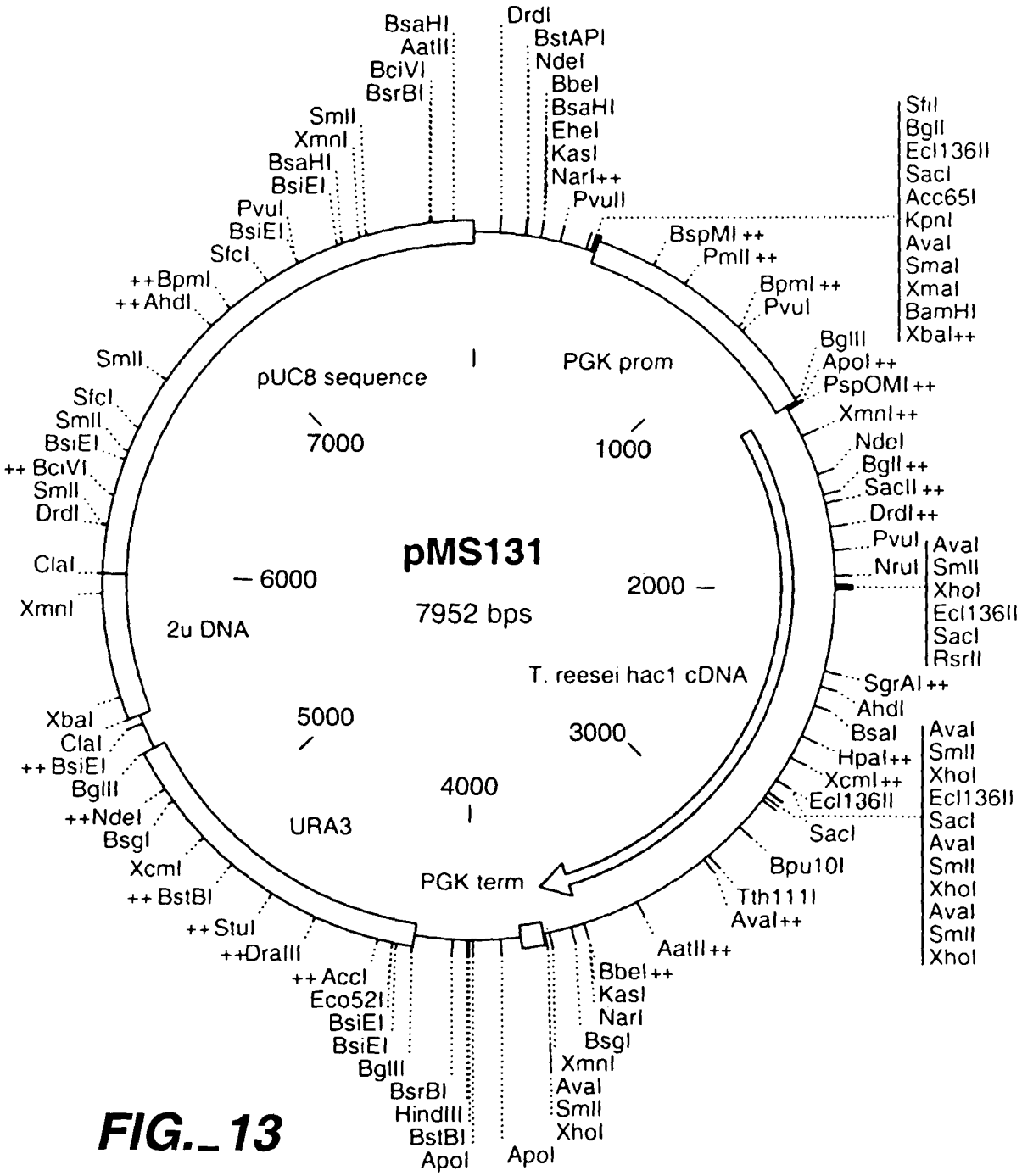

pMS131
7952 bps
pUC8 sequence
PGK prom
PGK term
T. reesei hac1 cDNA
2u DNA
URA3
1000
2000
3000
4000
5000
6000
7000
BsaHI
AatII
BciVI
BsrBI
SmlI
XmnI
BsaHI
BsiEI
PvuI
BsiEI
SfcI
++BpmI
++AhdI
SmlI
SfcI
SmlI
BsiEI
++BciVI
SmlI
DrdI
ClaI
XmnI
XbaI
ClaI
++BsiEI
BglII
++NdeI
BsgI
XcmI
++BstBI
++StuI
++DraIII
++AccI
Eco52I
BsiEI
BsiEI
BglII
BsrBI
HindIII
BstBI
ApoI
ApoI
XhoI
SmlI
AvaI
XmnI
BsgI
NarI
KasI
BbeI++
AatII++
Tth111I
AvaI++
Bpu10I
SacI
Ecl136II
XcmI++
HpaI++
BsaI
AhdI
SgrAI++
RsrII
SacI
Ecl136II
XhoI
SmlI
AvaI
NruI
PvuI
DrdI++
SacII++
BglI++
NdeI
XmnI++
PspOMI++
ApoI++
BglII
XbaI++
BamHI
XmaI
SmaI
AvaI
KpnI
Acc65I
SacI
Ecl136II
BglI
SfiI
PvuI
BpmI++
PmlI++
BspMI++
PvuII
NarI++
KasI
EheI
BsaHI
BbeI
NdeI
BstAPI
DrdI

FIG._13

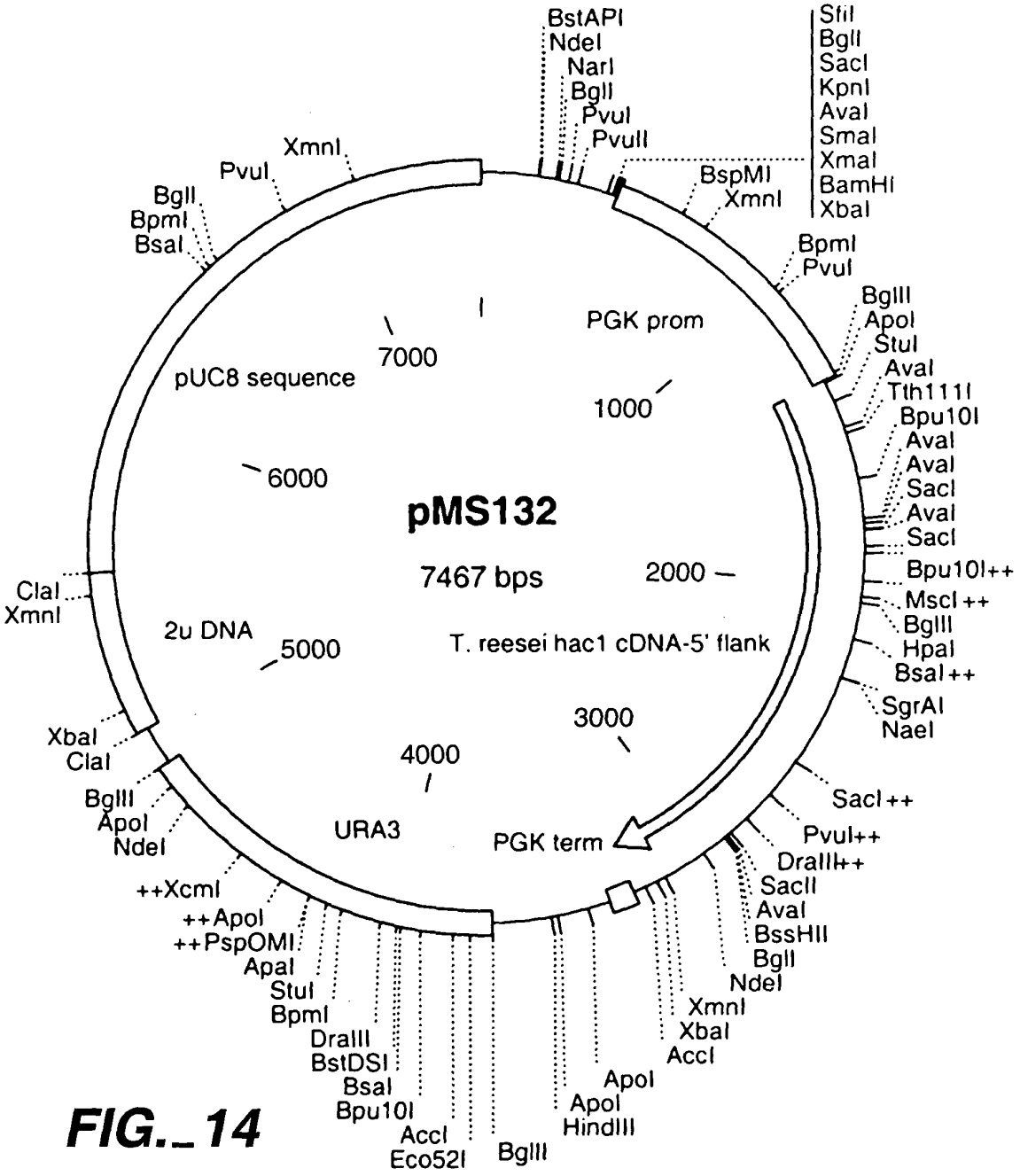

pMS132
7467 bps
PGK prom
PGK term
URA3
2u DNA
pUC8 sequence
T. reesei hac1 cDNA-5' flank
1000
2000
3000
4000
5000
6000
7000
BstAPI
NdeI
NarI
BglI
PvuI
PvuII
BspMI
XmnI
SfiI
BglI
SacI
KpnI
AvaI
SmaI
XmaI
BamHI
XbaI
BpmI
PvuI
BglII
ApoI
StuI
AvaI
Tth111I
Bpu10I
AvaI
AvaI
SacI
AvaI
SacI
Bpu10I++
MscI++
BglII
HpaI
BsaI++
SgrAI
NaeI
SacI++
PvuI++
DraIII++
SacII
AvaI
BssHII
BglI
NdeI
XmnI
XbaI
AccI
ApoI
ApoI
HindIII
BglII
Eco52I
AccI
Bpu10I
BsaI
BstDSI
DraIII
BpmI
StuI
ApaI
++PspOMI
++ApoI
++XcmI
NdeI
ApoI
BglII
ClaI
XbaI
XmnI
ClaI
2u DNA
BglII
BpmI
BsaI
PvuI
XmnI

FIG._14

FIG._15A

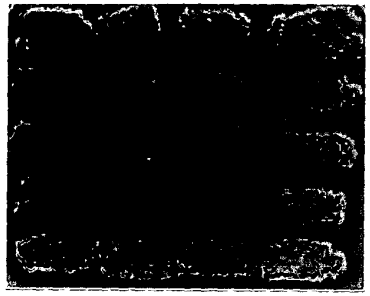


FIG._15B

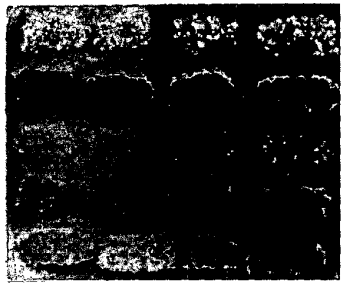
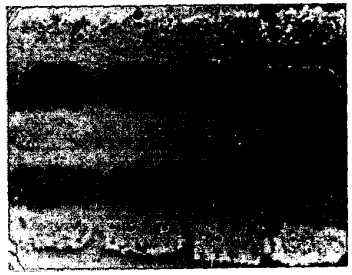
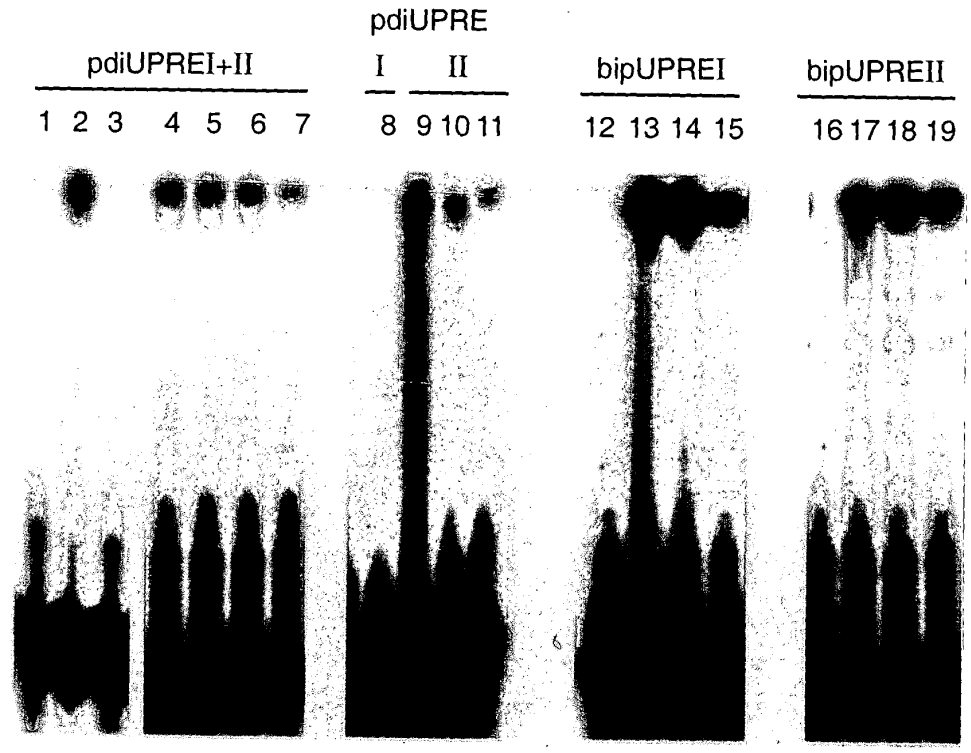


FIG._15C

FIG._15D






```
pdiUPREII    GCCAGATGTGGC
bipUPREI     GCCAA-CGTGTC
bipUPREII    GCGAGAAGTGGC
             ** *   *** *
```

FIG._16

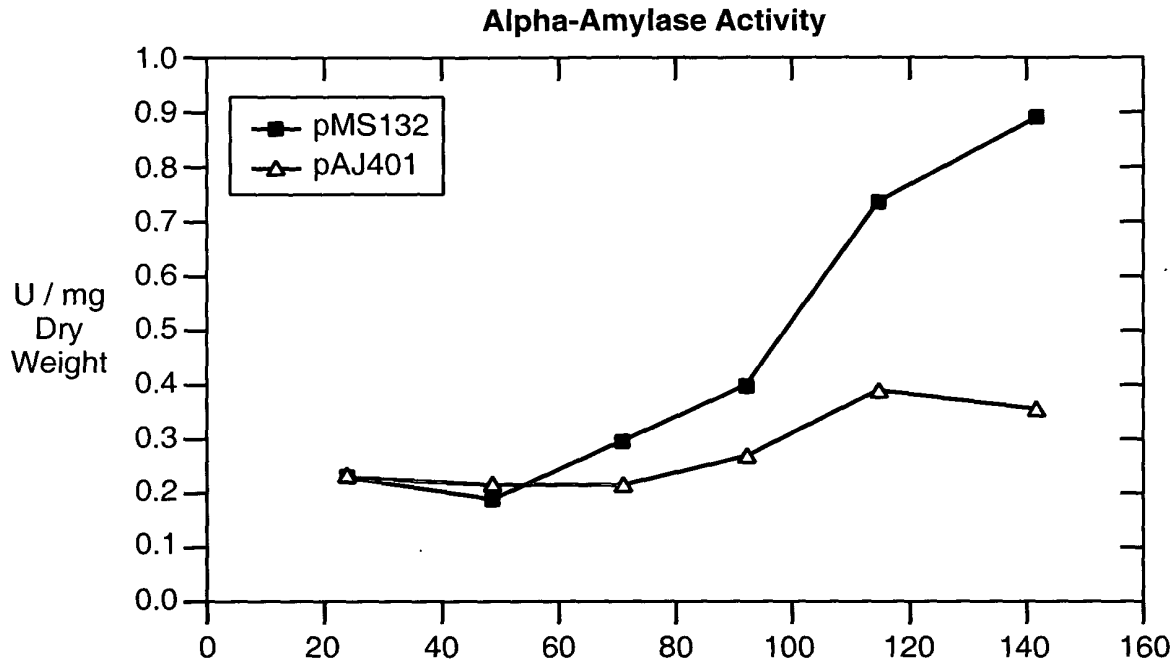

Alpha-Amylase Activity
pMS132
pAJ401
U / mg
Dry
Weight
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
20
40
60
80
100
120
140
160
Hours

FIG._17

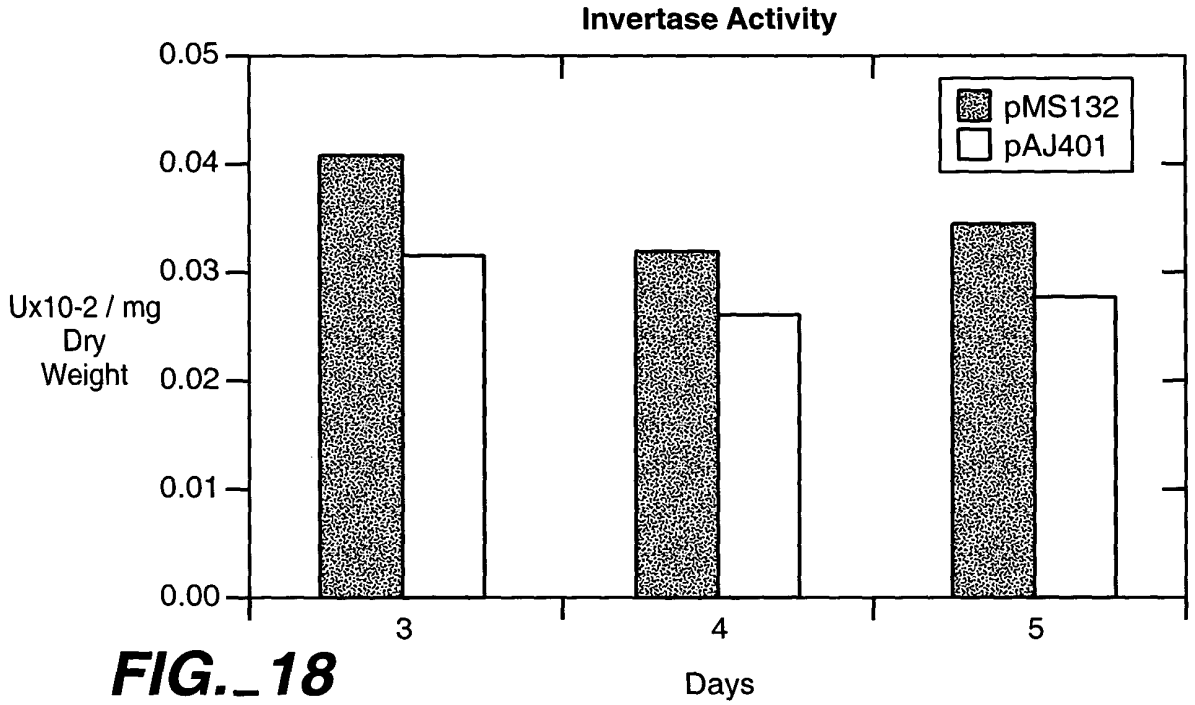

Invertase Activity
pMS132
pAJ401
Ux10-2 / mg
Dry
Weight
0.05
0.04
0.03
0.02
0.01
0.00
3
4
5
Days

FIG._18

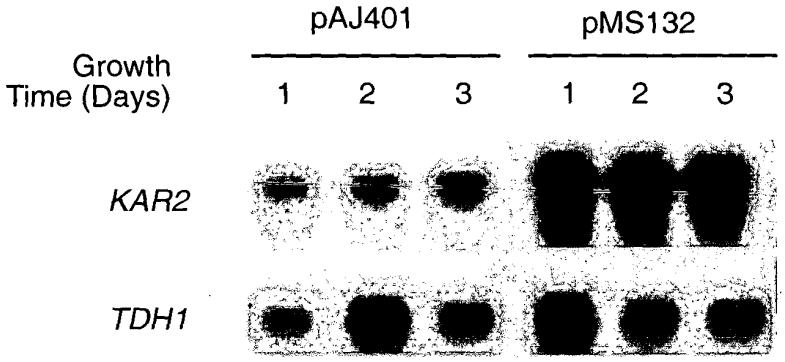


FIG._19A

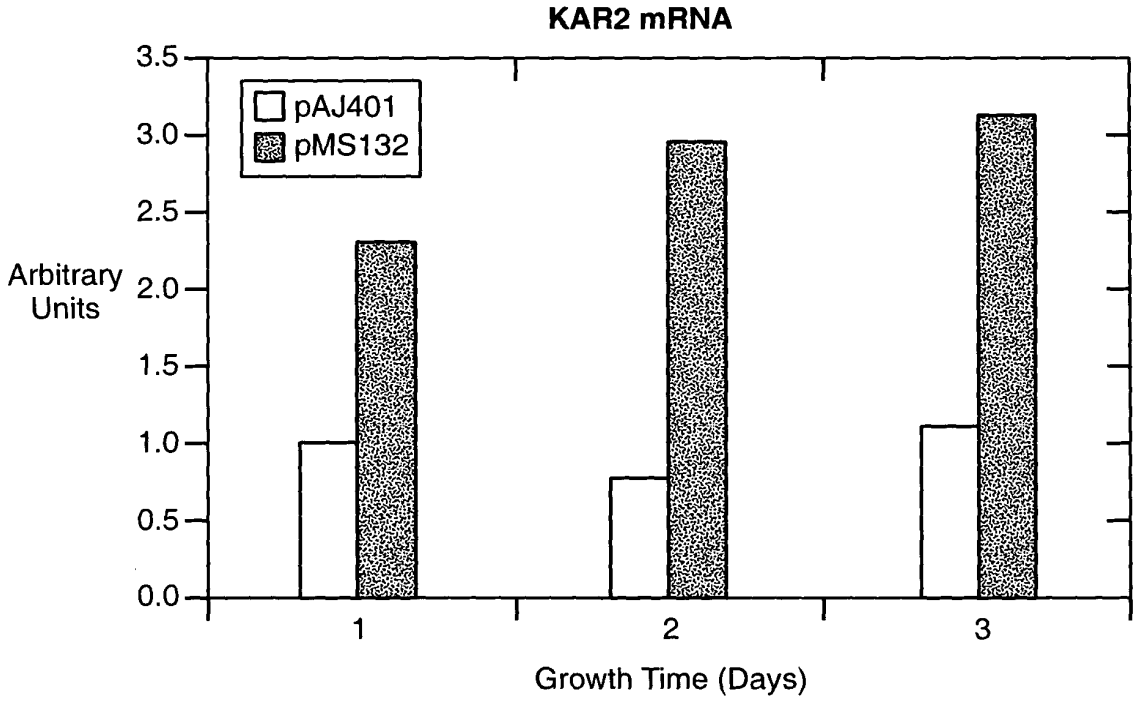


FIG._19B

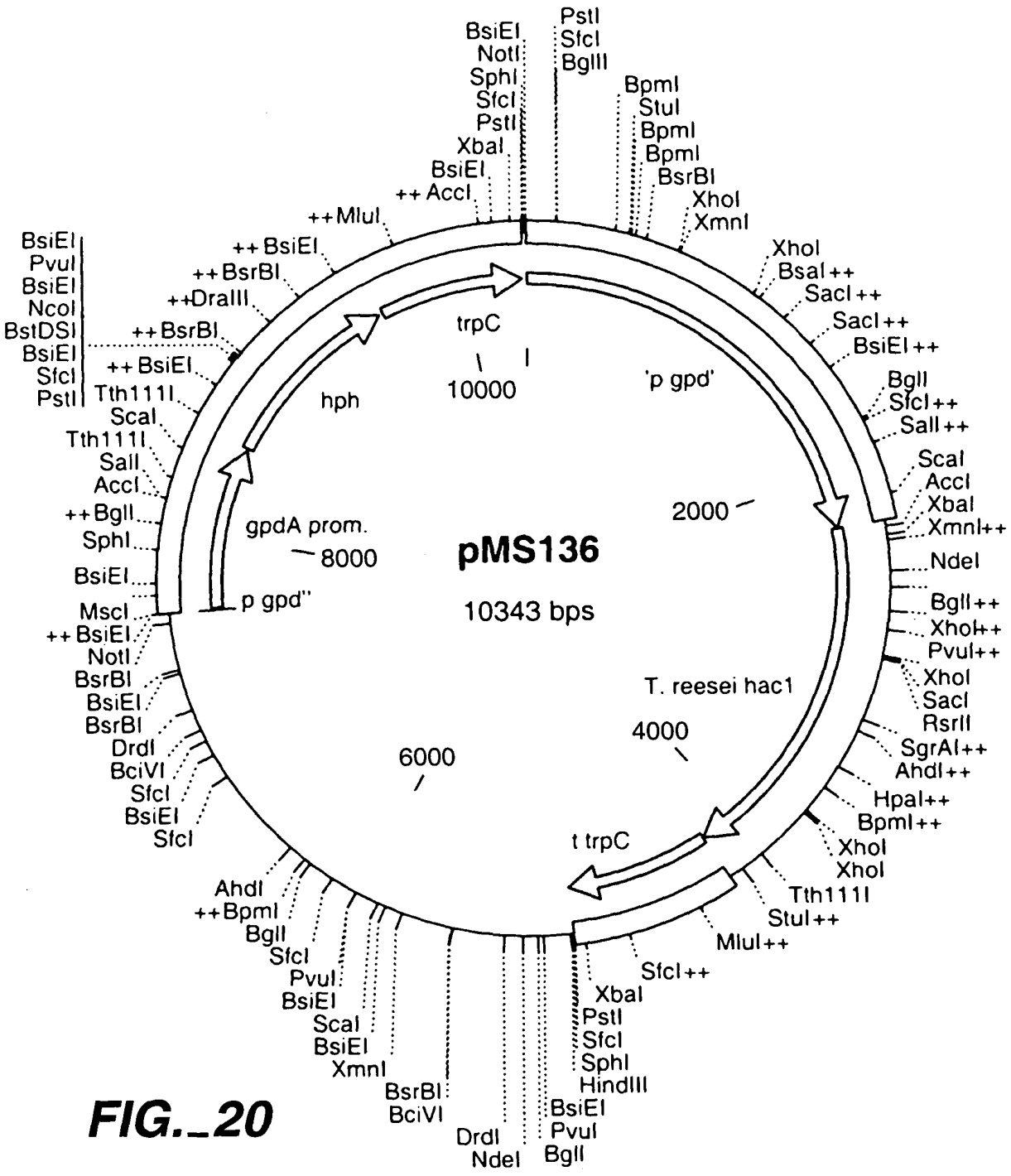

pMS136
10343 bps
trpC
10000
'p gpd'
hph
2000
gpdA prom.
8000
p gpd''
T. reesei hac1
4000
6000
t trpC
PstI
SfcI
BglII
BsiEI
NotI
SphI
SfcI
PstI
XbaI
BsiEI
++AccI
++MluI
++BsiEI
++BsrBI
++DraIII
++BsrBI
++BsiEI
Tth111I
ScaI
Tth111I
SalI
AccI
++BglI
SphI
BsiEI
MscI
++BsiEI
NotI
BsrBI
BsiEI
BsrBI
DrdI
BciVI
SfcI
BsiEI
SfcI
BsiEI
PvuI
NcoI
BstDSI
BsiEI
SfcI
PstI
BpmI
StuI
BpmI
BpmI
BsrBI
XhoI
XmnI
XhoI
BsaI++
SacI++
SacI++
BsiEI++
BglI
SfcI++
SalI++
ScaI
AccI
XbaI
XmnI++
NdeI
BglI++
XhoI++
PvuI++
XhoI
SacI
RsrII
SgrAI++
AhdI++
HpaI++
BpmI++
XhoI
XhoI
Tth111I
StuI++
MluI++
SfcI++
XbaI
PstI
SfcI
SphI
HindIII
BsiEI
PvuI
BglI
DrdI
NdeI
BsrBI
BciVI
XmnI
BsiEI
ScaI
BsiEI
PvuI
BglI
SfcI
AhdI
++BpmI

FIG._20

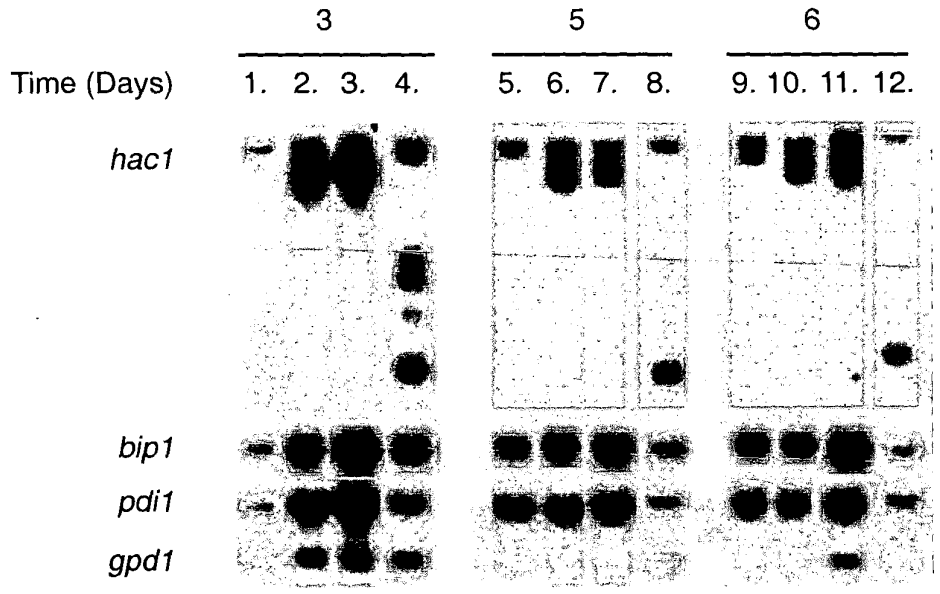


FIG._21A

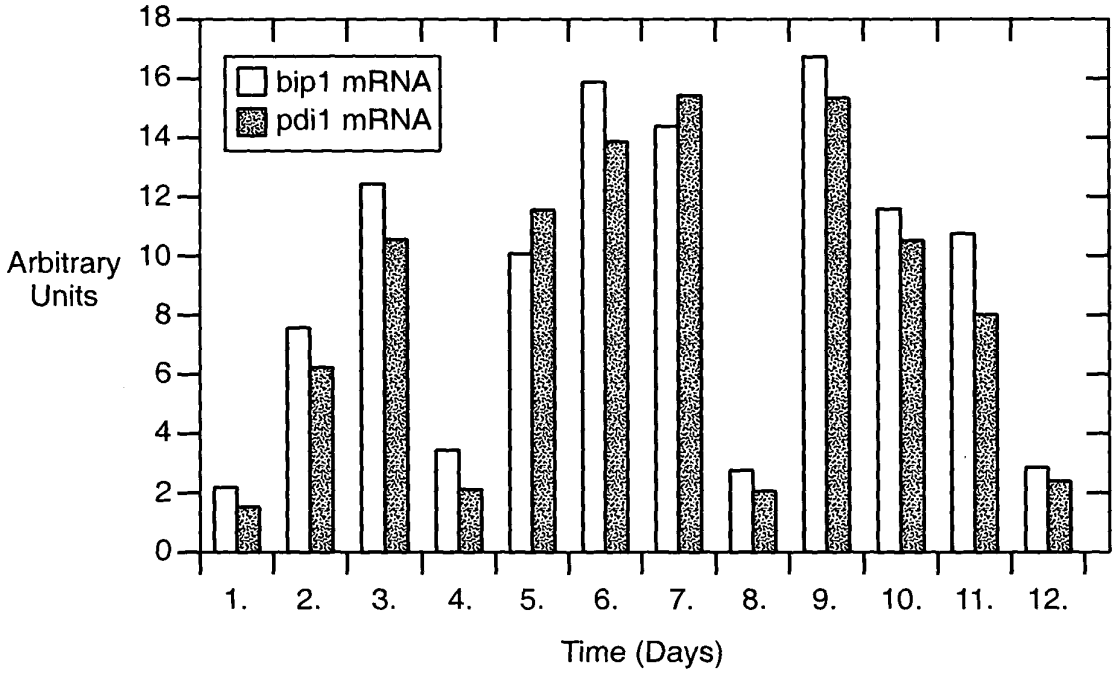


FIG._21B

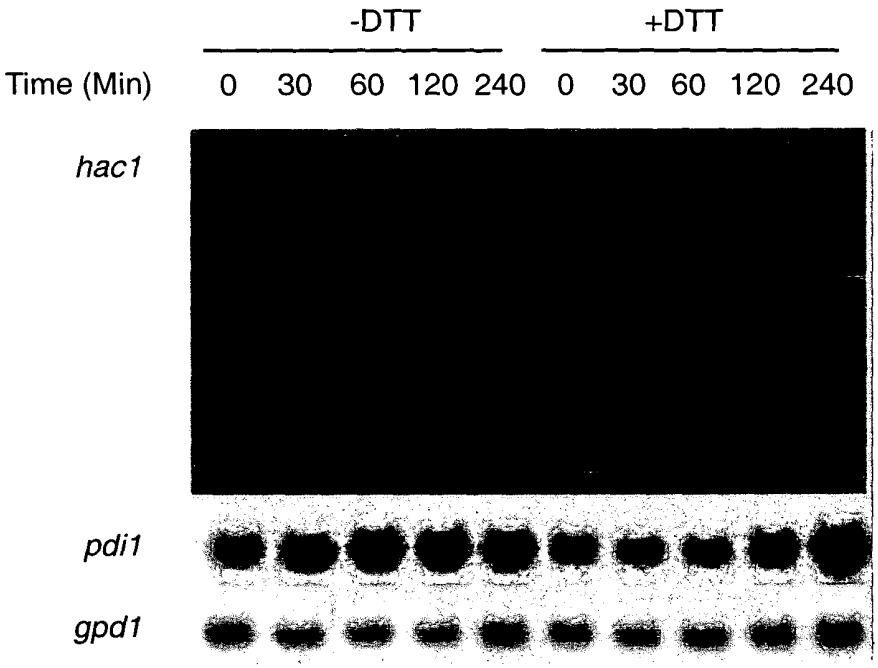


FIG._22A

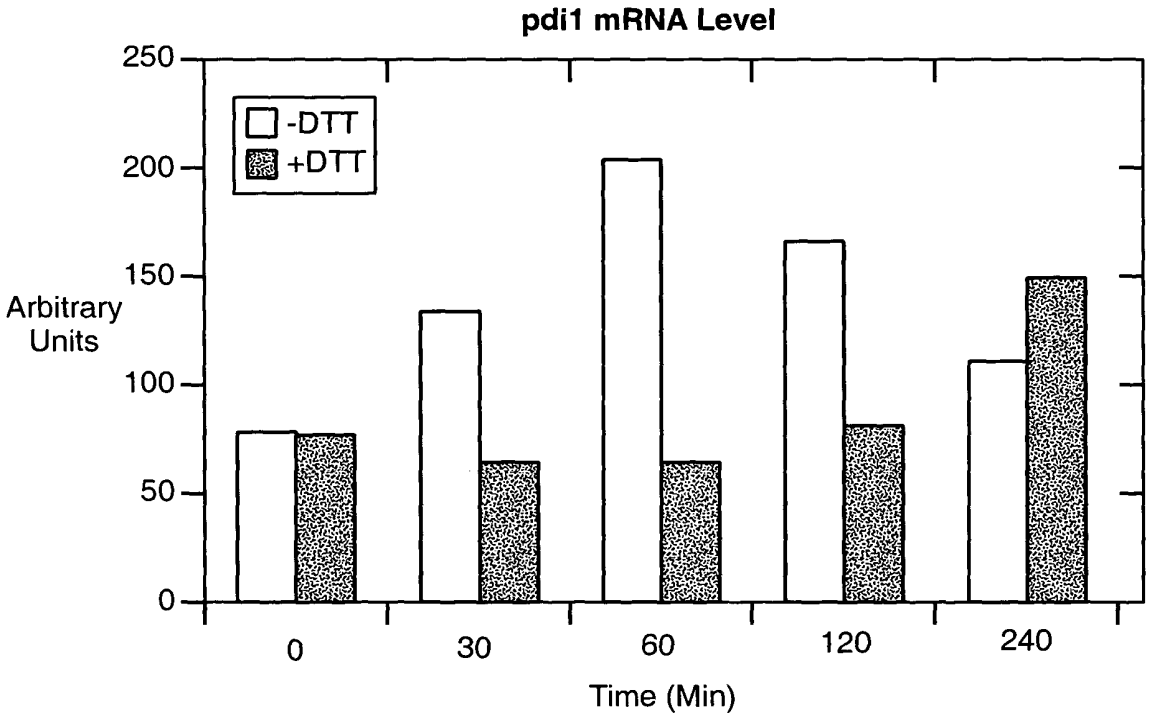


FIG._22B

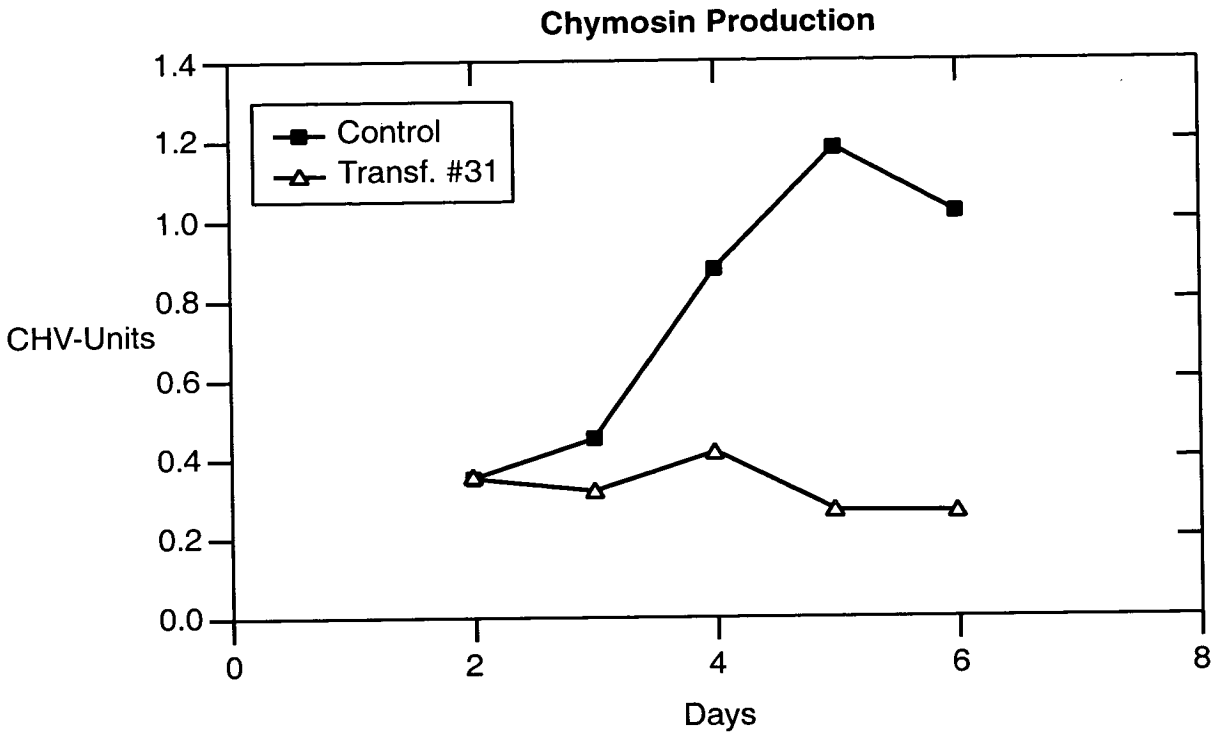

Chymosin Production
Control
Transf. #31
CHV-Units
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
Days

FIG._23

```
1     TTTGAACAGCAGATCGTTACTGCCTACCCAGACGTTACAGTCCACGAGCTCACGGAGGAC
      F  E  Q  Q  I  V  T  A  Y  P  D  V  T  V  H  E  L  T  E  D
61    GATGAATTCTTAGTAATCGCTTGCGATGgtgggtttcccctcaactttgccgctctgttc
      D  E  F  L  V  I  A  C  D  G
121   cacaatctgatatactacagGAATCTGGGATTGCCAGTCTTCCCAAGCCGTGGTCGAATT
                              I  W  D  C  Q  S  S  Q  A  V  V  E  F
181   CGTTCGCCGCGGTATCGCGGCCAAGCAGGATCTCTATCGGATTTGTGAAAACATGATGGA
       V  R  R  G  I  A  A  K  Q  D  L  Y  R  I  C  E  N  M  M  D
241   CAACTGTCTCGCTTCCAACAGTGAGACTGGTGGAGTTGGCTGTGACAACATGACAATGGT
       N  C  L  A  S  N  S  E  T  G  G  V  G  C  D  N  M  T  M  V
301   CATTATAGGTCTCCTCAATGGAAAAACTAAGGAAGAGTGGTACAACCAGATCGCGGAGCG
       I  I  G  L  L  N  G  K  T  K  E  E  W  Y  N  Q  I  A  E  R
361   GGTTGCTAACGGCGACGGCCCTTGTGCTCCGCCCGAATACGGCAAGTCTCTCGAGGAACC
       V  A  N  G  D  G  P  C  A  P  P  E  Y  G  K  S  L  E  E  P
421   CACGGCCTCCAATCCCTACTGACTGAACCGTGGGGGTTGCAGCTGAATTCCGAGGACCTG
       T  A  S  N  P  Y  *
481   GAATCCATAACCATTTTGAAGAGAACCCGGACGAGTACGAGATCGACCACGATCGCTCCC
541   GCCCATTCAACGTGCGTTCTGGTAGAATAATTCTTTTGGGAGATGGCAGCACGTTAATTC
601   CAGGAAAACAGAATGACGAGGAACTCTTTGACCAAACCGGGGAGGAGAATCACCCAGACC
661   AAGTGCAACGCCAGAATACCGACACAGAAAGAAATGACCGTGAAGGGACGCCTGGGCCTC
721   AATCCGCGGCTCCCCAGACGAACACGTCCGCTTCGGATGGCTCAGAGCCTTCTAACACAC
781   CGCAGAAACCCGCCTCTTCGTAGCTTCGTCATGAGATTTACGCCTGATTCCCTTCATTTT
841   GGTTCCTGAAACGACTCGTGATTTCACGATCCACACCCGCCGCCCCATCTCCACGCCCGG
901   TGCCGAAGCCTCACAATTCTGCCCCCATACGGTCGCTCATTGATTTTCTGTTTCTCACGA
961   TTTGAAGGCGCATTGGTGCTTGTGACCGCGAAGATGCGAAAGAGACGGACCATATCATCC
1021  CCTTCTATCTCTTGTTTTAATCCCATCTTCTTACTTTTTACGAGCTCATCCAGATCAAAT
1081  CACCTTCGTGTTACTCCAGGATGGATATCTTTGAGAATTCGCCGAATGGGTGGAGGCATC
1141  TTCTTTCCCTGTCATCTTTCTTCTCTATGTTTGCACATGCCGCAAGCGGCAGGCCTCACG
1201  AGAGTACGTTTGTTTCATGTCTCGACATAAGATACCGCAACAACCACTATTGACGAACTT
1261  TATAA
```

FIG._24

```
1          GACGAGCCTCGATCCGCCTCGACGCCGCTGGTTTCCCCCTTCTTTCTCCCCCCT
61    TCAGCCACGTCCTCGTGTCCTATAACCTTTCGCAGCCTACGGTCCCGCCTCCAGAGGTCT
121   CGCGTCCCTGAGTACCAAACGATAGAAACAAGACTGCTATCTTTGTCGTGCTGCCTCCTC
181   CCCTCCTCGACGCTTTTCCTCCCCCTCGATCGCTTTCCCGGCCCTCGTGAGACGTCGCAG
241   CCATGGGCCAAACCCTCTCGGAGCCCGTTGTCGAAAAGACTTCCGAAAAGGGCGAGGATG
        M  G  Q  T  L  S  E  P  V  V  E  K  T  S  E  K  G  E  D
301   ACAGACTCATCTACGGCGTGTCCGCCATGCAGGGCTGGCGCATCAGCATGGAGGACGCTC
      D  R  L  I  Y  G  V  S  A  M  Q  G  W  R  I  S  M  E  D  A
361   ACACGGCTGAGCTGAATCTCCCCCCACCTGACAACGACACCAAGACGCACCCCGACAGGC
      H  T  A  E  L  N  L  P  P  P  D  N  D  T  K  T  H  P  D  R
421   TGTCCTTTTTCGGAGTCTTCGACGGACACGGAGGAGACAAAGTAGCGTTATTCGCAGGCG
      L  S  F  F  G  V  F  D  G  H  G  G  D  K  V  A  L  F  A  G
481   AGAACATTCACAACATTGTTTTCAAGCAGGAGAGCTTCAAATCCGGTGATTACGCTCAGG
      E  N  I  H  N  I  V  F  K  Q  E  S  F  K  S  G  D  Y  A  Q
541   GTCTCAAGGACGGCTTTCTCGCTACGGATCGGGCTATTCTCAACGACCCCAAATACGAAG
      G  L  K  D  G  F  L  A  T  D  R  A  I  L  N  D  P  K  Y  E
601   AGGAAGTCTCTGGCTGCACTGCCTGCGTCACCCTGATTGCCGGAAACAAACTATATGTCG
      E  E  V  S  G  C  T  A  C  V  T  L  I  A  G  N  K  L  Y  V
661   CCAACGCCGGTGATTCTCGAAGCGTGCTGGGCATCAAGGGACGGGCCAAACCCCTATCCA
      A  N  A  G  D  S  R  S  V  L  G  I  K  G  R  A  K  P  L  S
721   ACGACCACAAGCCTCAGCTTGAAACGGAGAAGAACCGAATCACAGCCGCTGGCGGTTTCG
      N  D  H  K  P  Q  L  E  T  E  K  N  R  I  T  A  A  G  G  F
781   TCGACTTTGGCCGAGTCAACGGCAATCTGGCTCTGTCGCGTGCCATTGGCGACTTTGAAT
      V  D  F  G  R  V  N  G  N  L  A  L  S  R  A  I  G  D  F  E
841   TCAAGAAGAGCGCCGAGCTGTCCCCCGAAAACCAGATCGTTACCGCCTTTCCCGATGTCG
      F  K  K  S  A  E  L  S  P  E  N  Q  I  V  T  A  F  P  D  V
901   AGGTGCACGAGCTTACAGAGGAGGACGAGTTCCTGGTGATTGCCTGTGACGGTATCTGGG
      E  V  H  E  L  T  E  E  D  E  F  L  V  I  A  C  D  G  I  W
961   ATTGCCAATCTTCCCAGGCTGTTGTTGAGTTTGTGCGACGAGGCATCGCCGCCAAGCAGG
      D  C  Q  S  S  Q  A  V  V  E  F  V  R  R  G  I  A  A  K  Q
1021  ACCTTGACAAGATCTGCGAGAACATGATGGACAACTGCCTTGCGTCCAACTCAGAAACGG
      D  L  D  K  I  C  E  N  M  M  D  N  C  L  A  S  N  S  E  T
1081  GTGGCGTCGGCTGCGACAACATGACCATGGTCATCATCGGCTTCCTGCACGGCAAGACCA
      G  G  V  G  C  D  N  M  T  M  V  I  I  G  F  L  H  G  K  T
1141  AGGAGGAGTGGTATGACGAAATTGCCAAGAGAGTGGCCAACGGAGACGGCCCCTGTGCCC
      K  E  E  W  Y  D  E  I  A  K  R  V  A  N  G  D  G  P  C  A
1201  CCCCGGAATATGCCGAGTTCCGCGGTCCCGGCGTTCACCACAACTACGAAGACAGCGACA
      P  P  E  Y  A  E  F  R  G  P  G  V  H  H  N  Y  E  D  S  D
1261  GCGGCTACGACGTCGACGCCGACAGCGGCGGCAAGTTTAGCCTTGCCGGATCCCGGGGTC
      S  G  Y  D  V  D  A  D  S  G  G  K  F  S  L  A  G  S  R  G
1321  GCATCATCTTCCTGGGCGACGGCACCGAAGTCCTGACGGGCTCCGACGACACGGAGATGT
      R  I  I  F  L  G  D  G  T  E  V  L  T  G  S  D  D  T  E  M
1381  TTGACAATGCTGACGAGGACAAGGACCTTGCGAGCCAGGTGCCCAAGAGCTCCGGCAAGA
      F  D  N  A  D  E  D  K  D  L  A  S  Q  V  P  K  S  S  G  K
1441  CCGATGCAAAGGAGGAGACAGAGGCCAAGCCGGCACCAGAGGCGGAGTCGTCCAAACCCG
      T  D  A  K  E  E  T  E  A  K  P  A  P  E  A  E  S  S  K  P
1501  CGGATGGGTCGGAGAAGAAGCAAGACGAAAAGACACCCGAGGAGAGTAAGAAGGATTAGG
      A  D  G  S  E  K  K  Q  D  E  K  T  P  E  E  S  K  K  D  *
1561  TGGTCCTCTTGAATTCTTTGGGCTCGTCTCCTTGAAGCCCCGCGCTGGTGTTGTTGATGG
1621  CGTGTGTTTGTGTGTACGTGTGGCATAATTCTTTTTTCTTCCCATCACCGCTACTCAAAA
1681  AACCCCAGGCGTGAGGGCATTTTTAAATCGCATAGGGAGTGGGGGAGAGACGGGAGAGGC
1741  TCTGGAACGAAACATTCTGGGAGACAAGGCAGAGAGCGTAGGGGCGGTTTAGACATTGAG
1801  TGTTGCTCGTTAAAAAAAAAAAAAAAAAA
```

FIG._25

```
CGGAGGCAAGAGTCATAGACGCGGGAAGAAGAAAATTGAGAGTGAGAAAGAGGAATCTGA   60
 G  G  K  S  H  R  R  G  K  K  K  I  E  S  E  K  E  E  S  D
TCACGCCCCTGGCACCTTGCAACCCCCGGCTGGGCCCGATGCCGGGTTAGCTCTCACCCG  120
 H  A  P  G  T  L  Q  P  P  A  G  P  D  A  G  L  A  L  T  R
CACTGCATCTAATGAGGTGTTTGAAGCGGACGGTGTCATCCAGATTGGCCGTTTGAAGGT  180
 T  A  S  N  E  V  F  E  A  D  G  V  I  Q  I  G  R  L  K  V
CTTTACGGCTGACGTTCTGGGTCATGGAAGCCACGGGACAGTTGTTTACCGCGGGTCGTT  240
 F  T  A  D  V  L  G  H  G  S  H  G  T  V  V  Y  R  G  S  F
TGACGGCCGAGACGTCGCGGTCAAACGTATGCTGGTGGAGTTCTATGATATTGCATCGCA  300
 D  G  R  D  V  A  V  K  R  M  L  V  E  F  Y  D  I  A  S  H
CGAAGTGGGATTGTTGCAGGAAAGCGATGATCATAACAACGTTATCCGATGTTATTGCCG  360
 E  V  G  L  L  Q  E  S  D  D  H  N  N  V  I  R  C  Y  C  R
TGAGCAAGCCAAGGGTTTCTTCTACATCGCCCTTGAACTGTGTCCGGCTTCTTTGCAGGA  420
 E  Q  A  K  G  F  F  Y  I  A  L  E  L  C  P  A  S  L  Q  D
TGTGGTAGAACGACCAGACGCGTTCCCGCAGCTAGTCAATGGTGGCTTGGATATGCCGGA  480
 V  V  E  R  P  D  A  F  P  Q  L  V  N  G  G  L  D  M  P  D
CGTCTTGCGTCAAATTGTCGCCGGTGTCCGGTACCTACACTCTCTCAAAATCGTACACCG  540
 V  L  R  Q  I  V  A  G  V  R  Y  L  H  S  L  K  I  V  H  R
TGACTTGAAGCCTCAAAATATCCTGGTCGCCGCTCCTCGAGGCCGTATCGGTTCTCGGGC  600
 D  L  K  P  Q  N  I  L  V  A  A  P  R  G  R  I  G  S  R  A
CATCCGGCTTCTGATTTCGGACTTTGGCTTGTGCAAGAAACTTGAGGATAACCAGAGTTC  660
 I  R  L  L  I  S  D  F  G  L  C  K  K  L  E  D  N  Q  S  S
ATTCAGGGCAACCACGGCCCATGCTGCTGGTACTCCGGGTGGAGGGCTCCCGAACTGCTT  720
 F  R  A  T  T  A  H  A  A  G  T  P  G  G  G  L  P  N  C  L
GTGGATGACGACAAGAGCCGGTAATCAGGGTTCAGAGTCTCAAAATACGGAGTCATCTGA  780
 W  M  T  T  R  A  G  N  Q  G  S  E  S  Q  N  T  E  S  S  E
GCCGGCGGTCGTCGATCCCCAGACGAATCGACGAGCCACCCGAGCCATTGATATCTTCTC  840
 P  A  V  V  D  P  Q  T  N  R  R  A  T  R  A  I  D  I  F  S
CCTGGGATGTGTCTTCTACTACGTCCTAACTCGAGGATGTCATCCTTTTGACAAGAATGG  900
 L  G  C  V  F  Y  Y  V  L  T  R  G  C  H  P  F  D  K  N  G
CAAGTTCATGCGCGAAGCAAATATCGTCAAGGGGAATTTCAATCTCGATGAGTTACAGCG  960
 K  F  M  R  E  A  N  I  V  K  G  N  F  N  L  D  E  L  Q  R
TCTAGGAGAGTATGCGTTTGAAGCAGACGATCTTATCCGATCAATGTTGGCACTTGATCC 1020
 L  G  E  Y  A  F  E  A  D  D  L  I  R  S  M  L  A  L  D  P
ACGTCAACGgtatgtcccaacaacatcttcctttgccttgtggcgtagcgtactaatctc 1080
 R  Q  R
cacagCCCCGACGCAAGCGCTGTGTTAACCCATCCTTTCTTCTGGAATCCGTCCGACCGC 1140
      P  D  A  S  A  V  L  T  H  P  F  F  W  N  P  S  D  R
CTTAGCTTCCTCTGTGACGTTTCGGACCACTTCGAGTTCGAACCGAGAGATCCTCCATCT 1200
L  S  F  L  C  D  V  S  D  H  F  E  F  E  P  R  D  P  P  S
GACGCTCTTCTGTGTCTAGAGTCTGTAGCCTCTGATGTCATTGGCCCTGAAATGAATCCT 1260
D  A  L  L  C  L  E  S  V  A  S  D  V  I  G  P  E  M  N  P
CAAACTCCTGCCAAAGGACTTCAAAGACAGTCTCGGAAGCAGCGAAAATACACCGGCTCC 1320
Q  T  P  A  K  G  L  Q  R  Q  S  R  K  Q  R  K  Y  T  G  S
AAAATGCTGGACTTGATGCGAGCCCTGCGGAACAAGCGCAACCACTACAATGATATGCCG 1380
K  M  L  D  L  M  R  A  L  R  N  K  R  N  H  Y  N  D  M  P
GAGCATTTGAAAGCTCATATTGGTGGGCTGCCGGAGGGTTACTTGAATTTCTGGACCGTG 1440
E  H  L  K  A  H  I  G  G  L  P  E  G  Y  L  N  F  W  T  V
CGTTTCCCGAGTTTGCTGATGAGTTGTCATTGGGTGATTGTTGAACTGGGATTGACGAAG 1500
R  F  P  S  L  L  M  S  C  H  W  V  I  V  E  L  G  L  T  K
ACGGATCGGTTCCAAGAGATATTTTACGCCATTGGAGTAGGTTGTTGCGTACTGGTTCAG 1560
T  D  R  F  Q  E  I  F  Y  A  I  G  V  G  C  C  V  L  V  Q
AAATATATTG
K  Y  I
```

FIG._26

```
1     GCACGAGCAAGATACGGCCTCTCGCACCAAGGAGACACGCATATTCGTGGTACCATCGGC
61    TGAGGGTGAAGGGGGGTTCAACACAGCACAACTCAGCGACCACTGGACTGGTGGAGCCGA
121   AGCCCACGATCGAATCCACAGCCTGCACCACTTTCTCCTCGTCATATTCGCGGGGACTCA
181   CAAGCGGTTTCCGTTGCCTTCGAATTCGACAGAGCTGCGACTGCGAGTCATTTCAGCGAC
241   TCTAAACCTACTCCTTTGGCTGCTGCGCGGGACTGGTTCTGCCCAGCCTCTCCTACTCGA
301   CCAACCGACGTCCTCTTTCTGCTTCCTCATCCCTTTCTCCTTTGACGTCCGAGCGTCAGA
361   GCGAATTTTTCCTTGCTTCTTCGTTTGGGCCGGGAATGGCTTCTCTGGCATCGCAACAGC
421   CTCTACCTCTCCGTTGGTAGAGCCATAGCCTGCAGCTCCCCATGTGATCCGCTCTCCGTC
481   TCTCCGGCACCCCGACTTTCGTCTCGATCATGATGCGGCGACCCCCGAGCCAAGGACGAT
                                                              M
541   GGTCCGCGTCGCATCAGAAGCTCTCCTGGCTTTTGCCTTTATTCTCATACCATGGCTCCA
       V  R  V  A  S  E  A  L  L  A  F  A  F  I  L  I  P  W  L  Q
601   ACTTGCCGATGCTCAGCAGCAGCCTCAGCAGCCCCAGATTCGAATTCACTCACAAAGAGG
       L  A  D  A  Q  Q  Q  P  Q  Q  P  Q  I  R  I  H  S  Q  R  G
661   CGACGCGCCCCTTGACAAAGTCGCCGACGATGCCAACACCCGTTGGTACGCAACACATGC
       D  A  P  L  D  K  V  A  D  D  A  N  T  R  W  Y  A  T  H  A
721   TGCACCAGACGTGCACCCCGAAGCGAAGTTCGACACCGTCAACAGGAAGCAAAAGCAGCA
       A  P  D  V  H  P  E  A  K  F  D  T  V  N  R  K  Q  K  Q  Q
781   GTCGACCGCTTCGCCCCAGCAACACCAGAAATATCGACGAGCCCCCTATGACTACGCCAG
       S  T  A  S  P  Q  Q  H  Q  K  Y  R  R  A  P  Y  D  Y  A  S
841   CAAGGACAAGGCCCAGAACCGATATGCGCAGCACCCTATCCGCGAATCCGAGAAACCAAA
       K  D  K  A  Q  N  R  Y  A  Q  H  P  I  R  E  S  E  K  P  N
901   CTACGTAAAAGTCCCCAACGATGCGAGCGCCCTCGCAACTTTAGCTCCGGCTCAGCCCGT
       Y  V  K  V  P  N  D  A  S  A  L  A  T  L  A  P  A  Q  P  V
961   CCGAGCACCACACACCTCACGACATCACTGGCCCAGCAGCAGCGCCGCTTCTGGGCTGGC
       R  A  P  H  T  S  R  H  H  W  P  S  S  S  A  A  S  G  L  A
1021  CTCGCCGCACAATGCGCGGAGTCTGGAGGACTGGGAAGTTGAAGACTTTGTTCTTCTGGC
       S  P  H  N  A  R  S  L  E  D  W  E  V  E  D  F  V  L  L  A
1081  GACCGTCGATGGAGACCTCTATGCCAGCGACCGAAAGACCGGTCGGCACCTCTGGCACCT
       T  V  D  G  D  L  Y  A  S  D  R  K  T  G  R  H  L  W  H  L
1141  CGAGGTCGACCAGCCAGTGGTTGAAACCAAACACTACCGAACAAACAACTCCGTCCTCGA
       E  V  D  Q  P  V  V  E  T  K  H  Y  R  T  N  N  S  V  L  D
1201  CGACGACTATCGCCCCGTCGACCACTACATCTGGGCCGTCGAGCCGAGCCGCGATGGAGG
       D  D  Y  R  P  V  D  H  Y  I  W  A  V  E  P  S  R  D  G  G
1261  GCTCTATGTATGGATCCCCGACTCCGGAGCGGGCCTCGTCAGGACCGGCTTCACCATGAA
       L  Y  V  W  I  P  D  S  G  A  G  L  V  R  T  G  F  T  M  K
1321  GCACCTCGTTGAAGAACTTGCTCCATACGCCGGCGACGAGCCCCCCGTTGTCTATACCGG
       H  L  V  E  E  L  A  P  Y  A  G  D  E  P  P  V  V  Y  T  G
1381  AGACAAGAAGACGACCATGGTCACCCTGGACGCCGCTACCGGGCGCGTTCTCAAATGGTT
       D  K  K  T  T  M  V  T  L  D  A  A  T  G  R  V  L  K  W  F
1441  TGGCTCTAGCGGCTCCCAAGTCAACGAAGCCGAGAGCTGCCTTCGGCCCAATGCCTTTGA
       G  S  S  G  S  Q  V  N  E  A  E  S  C  L  R  P  N  A  F  D
1501  CGACAGGGATACCACAGAGTGCAGCTCCATGGGCACAATCACGCTGGGAAGGACCGAGTA
       D  R  D  T  T  E  C  S  S  M  G  T  I  T  L  G  R  T  E  Y
```

FIG._27A

```
1561  CACGGTGGGCATCCAGAGGCGAGACGGTCGCCCTATCGCAACCTTGAAGTACGCAGAATG
        T  V  G  I  Q  R  R  D  G  R  P  I  A  T  L  K  Y  A  E  W
1621  GGGACCCAACACCTTTGACAGCGACCTCTACCAGCAATACCACGCCTCGTTGGACAACCA
        G  P  N  T  F  D  S  D  L  Y  Q  Q  Y  H  A  S  L  D  N  H
1681  TTACATCACCAGTCAGCACGACGGGAGAATTTACGCGTTTGACAAGTCACAGGCAGAAAA
        Y  I  T  S  Q  H  D  G  R  I  Y  A  F  D  K  S  Q  A  E  N
1741  CGACCTGCCCCTCTACACCCACAAGTTTTCGTCTCCCGTCGCCCGGGTCTTCGATGTCTG
        D  L  P  L  Y  T  H  K  F  S  S  P  V  A  R  V  F  D  V  C
1801  TCGACCGTGGGATGCGAATGCGGGAAGCAACCCGGAGCTGGTGGTTCTCCCCCAACCTCC
        R  P  W  D  A  N  A  G  S  N  P  E  L  V  V  L  P  Q  P  P
1861  AATTCCAGCGCTTGATGAGAGCACTGTCAAGATGCGAAGCAACAGCATCTTCCTCAACCA
        I  P  A  L  D  E  S  T  V  K  M  R  S  N  S  I  F  L  N  Q
1921  GACTGAAAGCGGCGACTGGTATGCGCTCTCCGGCCGTGCGTATCCGCTTATACTCGATGC
        T  E  S  G  D  W  Y  A  L  S  G  R  A  Y  P  L  I  L  D  A
1981  CCCCGTGGCCCAGATCTCGCGGGACGACTTGTGGGATATGGCCCATGCCTTTGATTCCAT
        P  V  A  Q  I  S  R  D  D  L  W  D  M  A  H  A  F  D  S  I
2041  TAACCCAAATAAGCTGTCCAAGGCCCTGGTGGGAACCCACTTTCTGAATCCCGTCAAGAG
        N  P  N  K  L  S  K  A  L  V  G  T  H  F  L  N  P  V  K  S
2101  CACCGGTTACCATCAGCCGCCGACGCTCCCTGCCGGCGCCCTCGACGAGTATTACGAGGA
        T  G  Y  H  Q  P  P  T  L  P  A  G  A  L  D  E  Y  Y  E  D
2161  CTTGGAGAACGCCTCAAACAATGCTCACGCCGTGACAAACACTGTTCCGGAGGAGCCCAC
        L  E  N  A  S  N  N  A  H  A  V  T  N  T  V  P  E  E  P  T
2221  CATCATCACCAAAGTCAAGGCTCTTCCGCAGAGTGCTGCGAACAGCGTCATTGACTTTGT
        I  I  T  K  V  K  A  L  P  Q  S  A  A  N  S  V  I  D  F  V
2281  CAGCAACCCCATTCTCATCATTTTCTTGATAGGCTCCTTGATCTACAACGAAAAGAAGCT
        S  N  P  I  L  I  I  F  L  I  G  S  L  I  Y  N  E  K  K  L
2341  GCGACGGTCGTATCATCGGTTCCGGACTCATGGCACAATCAAGGACGTCTATCCCTTCTT
        R  R  S  Y  H  R  F  R  T  H  G  T  I  K  D  V  Y  P  F  F
2401  CGTTATCGAATCTGAGGCCGGAGATGAATCAGGTGATGACAAGGACGGTGTGTTCCCATC
        V  I  E  S  E  A  G  D  E  S  G  D  D  K  D  G  V  F  P  S
2461  TTCGCCGTCTCCGCGCAGTCAACCCCAGGACCAAAATGCGGAAGACCACCTGTCCAGACA
        S  P  S  P  R  S  Q  P  Q  D  Q  N  A  E  D  H  L  S  R  H
2521  CAAGGTGGAGAGGAATGCCGGCGACCAGGACAAGGTCAAGGACAACAGGAGCCTGCATGA
        K  V  E  R  N  A  G  D  Q  D  K  V  K  D  N  R  S  L  H  D
```

FIG._27B

```
2581  CGTTTCTGACACCTTGGAACCGAGCAACAAGACTGTTGAGAAAACGGCCGATGTGGTCAA
        V  S  D  T  L  E  P  S  N  K  T  V  E  K  T  A  D  V  V  K
2641  GCAAGTGGATGTAGCTGGCCCTGACGCACCCTCGACGGACTCCAATGGTGCTGCACCGGA
        Q  V  D  V  A  G  P  D  A  P  S  T  D  S  N  G  A  A  P  E
2701  GAAGAAGAAGAAGGCTCACCGAGGCCGTCGTGGCGGTGTCAAGCACAGAAAGGGTCGGCC
        K  K  K  K  A  H  R  G  R  R  G  G  V  K  H  R  K  G  R  P
2761  CACCGACGGCTCGCAGTCTCATGAAAACGACCCAGCTCTCACTACAGTGGACGAGGCTGT
        T  D  G  S  Q  S  H  E  N  D  P  A  L  T  T  V  D  E  A  V
2821  AAGCAATGCGAAGAAGCTGGGTGACCGGCCAAGCCTGGAACCCGACGTCATGACCATCTA
        S  N  A  K  K  L  G  D  R  P  S  L  E  P  D  V  M  T  I  Y
2881  CAACGACATGCAAGCCGTCACGGGCTCTGTTATCAGCATGGGAAACATCGAGGTCGATAC
        N  D  M  Q  A  V  T  G  S  V  I  S  M  G  N  I  E  V  D  T
2941  GGATGTCGAGCTTGGCATGGGCAGCAACGGTACTGTCGTATTTGCTGGCCGATTCGATGG
        D  V  E  L  G  M  G  S  N  G  T  V  V  F  A  G  R  F  D  G
3001  CAGGGACGTCGCCGTCAAGAGAATGACGATTCAGTTCTACGACATTGCCACGCGAGAAAC
        R  D  V  A  V  K  R  M  T  I  Q  F  Y  D  I  A  T  R  E  T
3061  TAAGTTGCTGCGCGAGAGTGACGACCACCCCAATgtaaatcagccctcatcgtttcaccc
        K  L  L  R  E  S  D  D  H  P  N
3121  attttcccttcgctaacgtaaccactgtctgcacGTCATTCGGTATTACTCACAAGTGCA
                                          V  I  R  Y  Y  S  Q  V  Q
3181  GCGAGGCGACTTCCTGTATATTGCCTTGGAACGCTGCGCTGCTTCATTGGCAGATGTCAT
        R  G  D  F  L  Y  I  A  L  E  R  C  A  A  S  L  A  D  V  I
3241  TGAAAAGCCGTATGCCTTTGGTGAATTGGCCAAGGCTGGACAAAAGGACCTACCGGGCGT
        E  K  P  Y  A  F  G  E  L  A  K  A  G  Q  K  D  L  P  G  V
3301  CTTGTACCAAATCACCAACGGCATCAGCCACTTGCACTCTCTGCGGATTGTTCATCGAGA
        L  Y  Q  I  T  N  G  I  S  H  L  H  S  L  R  I  V  H  R  D
3361  CTTGAAGCCTCAAAACATCTTGGTCAACTTGGACAAGGACGGCAGACCAAGGCTCTTGGT
        L  K  P  Q  N  I  L  V  N  L  D  K  D  G  R  P  R  L  L  V
3421  GTCGGACTTTGGCCTGTGTAAGAAACTGGAGGATAGACAGTCTTCGTTCGGAGCAACGAC
        S  D  F  G  L  C  K  K  L  E  D  R  Q  S  S  F  G  A  T  T
3481  AGGCCGAGCCGCTGGAACGTCGGGATGGCGTGCCCCCGAACTGCTTCTCGATGACGACGG
        G  R  A  A  G  T  S  G  W  R  A  P  E  L  L  L  D  D  D  G
3541  ACAGAATCCCGCAGCCATCGATAGCAGTACGCACAGCGGCTCTCACACCATCCTCGTGGG
        Q  N  P  A  A  I  D  S  S  T  H  S  G  S  H  T  I  L  V  G
3601  AGACCCCAACTCGCTTTCCAATGGAGGGCGAGCCACGAGGGCCATTGACATCTTCTCCCT
        D  P  N  S  L  S  N  G  G  R  A  T  R  A  I  D  I  F  S  L
3661  TGGCCTTGTCTTCTTCTACGTGCTCACCAATGGATCCCACCCGTTTGACTGTGGCGACAG
        G  L  V  F  F  Y  V  L  T  N  G  S  H  P  F  D  C  G  D  R
3721  ATATATGCGGGAGGTGAACATTCGAAAGGGCAACTACAATCTCGATCCATTGGACGCTCT
        Y  M  R  E  V  N  I  R  K  G  N  Y  N  L  D  P  L  D  A  L
3781  GGGCGACTTTGCCTACGAAGCCAAGGATCTGATTGCGTCCATGCTCCAGGCCTCTCCCAA
        G  D  F  A  Y  E  A  K  D  L  I  A  S  M  L  Q  A  S  P  K
3841  GGCACGACCCGACTCGCGAGAGGTCATGGCCCACCCTTTCTTCTGGTCTCCGAAGAAGCG
        A  R  P  D  S  R  E  V  M  A  H  P  F  F  W  S  P  K  K  R
3901  TCTGGCCTTTTTGTGCGACGTGTCGGATTCTCTGGAGAAGGAGGTGCGAGATCCTCCGTC
        L  A  F  L  C  D  V  S  D  S  L  E  K  E  V  R  D  P  P  S
3961  GCCTGCCTTGGTCGAGCTGGAGCGACATGCGCCGGAGGTCATTAAGGGAGACTTCTTGAA
        P  A  L  V  E  L  E  R  H  A  P  E  V  I  K  G  D  F  L  K
4021  GGTGCTCACGCGCGACTTTGTCGAGTCGCTGGGCAAGCAGCGCAAGTACACCGGGAACAA
        V  L  T  R  D  F  V  E  S  L  G  K  Q  R  K  Y  T  G  N  K
4081  GCTGCTCGACCTGTTGCGCGCTCTTCGCAACAAGCGGAATCACTACGAAGACATGTCGGA
        L  L  D  L  L  R  A  L  R  N  K  R  N  H  Y  E  D  M  S  D
4141  CTCGCTGAAGCGCAGCGTGGGATCACTGCCTGATGGGTATCTTGCTTATTGGACGGTCAA
        S  L  K  R  S  V  G  S  L  P  D  G  Y  L  A  Y  W  T  V  K
4201  GTTCCCGATGCTGTTGCTGACGTGCTGGAACGTGGTGTATAATCTCGAGTGGGAGAAGAC
        F  P  M  L  L  L  T  C  W  N  V  V  Y  N  L  E  W  E  K  T
4261  GGATCGGTTCAGGGAGTACTATGAGCCTGCCGGATTGTAGAAGAAAGAAAAGGAAGAGAA
        D  R  F  R  E  Y  Y  E  P  A  G  L  *
4321  AAGAAAGGCCTCTTGCTTGTTTGGTTGCTGTATATCTTTTTGCTCGAAGATGGAAACGGA
4381  AAATATTGGGGAAGTTGCATGGGAAGTGAACAAAAGAGGGGAAAAATGGTGAATGTGAAA
4441  GCAAAGTCGGTTAGCGGGTGGGCATGGTCGTCATCCATGTAATTGTTTCAGCTTCTGTTG
4501  CATCAAAAGCGTTGTGTTTTCGTTCTTT
```

FIG._27C

```
1      CTTTTTATTGTTCTATGGTTCTTAAGGACACCTGTCCTTCTTGGCCCTATCCTTCTTGTT
                     M  V  L  K  D  T  C  P  S  W  P  Y  P  S  C

61     GTCTGGTACACTTGACCCCAGGCACCACTTGGCCAGGCCTGGCCCCCCCAGCTTCCCCCG
       C  L  V  H  L  T  P  G  T  T  W  P  G  L  A  P  P  A  S  P

121    TTATGACACGGTGGCCTGTGTTCCTGTGACACGGGCAAGCAGACGTCCTCCACAAGCTGT
       V  M  T  R  W  P  V  F  L

181    GTCGACCTACATCACCGTCCTCCCTTGCAGTGCGGTTAAGATAAGGCTCATAGTAAATCG
241    ATTGATCCACAATTAAAGATCAATCACCTGTCACGCTTGAAATGATGGAAGAAGCATTCT
                                                  M  M  E  E  A  F

301    CTCCAGTCGACTCCCTCGCCGGCTCCCCGACGCCTGAGTTGCCATTGTTGACAGTGTCCC
       S  P  V  D  S  L  A  G  S  P  T  P  E  L  P  L  L  T  V  S

361    CGGCGGACACGTCGCTTGATGACTCGTCAGTACAGGCAGGGGAGACCAAGGCGGAAGAGA
       P  A  D  T  S  L  D  D  S  S  V  Q  A  G  E  T  K  A  E  E

421    AGAAGCCTGTGAAGAAGAGAAAGTCATGGGGCCAGGAATTGCCAGTCCCGAAGACTAACT
       K  K  P  V  K  K  R  K  S  W  G  Q  E  L  P  V  P  K  T  N

481    TGCCCCCAAGGAAACGGGCCAAGACTGAAGATGAGAAAGAGCAACGTCGTATCGAGCGCG
       L  P  P  R  K  R  A  K  T  E  D  E  K  E  Q  R  R  I  E  R
```

FIG._28A

```
541   TTCTTCGCAATCGTGCGGCAGCACAAACATCACGCGAGCGCAAGAGGCTCGAAATGGAGA
      V  L  R  N  R  A  A  A  Q  T  S  R  E  R  K  R  L  E  M  E

601   AGTTGGAAAATGAGAAGATTCAGATGGAACAGCAAAACCAGTTCCTTCTGCAACGACTAT
      K  L  E  N  E  K  I  Q  M  E  Q  Q  N  Q  F  L  L  Q  R  L

661   CCCAGATGGAAGCTGAGAACAATCGCTTAAACCAACAAGTCGCTCAACTATCTGCTGAGG
      S  Q  M  E  A  E  N  N  R  L  N  Q  Q  V  A  Q  L  S  A  E

721   TCCGGGGCTCCCGTGGCAACACTCCCAAGCCCGGCTCCCCCGTCTCAGCTTCTCCTACCC
      V  R  G  S  R  G  N  T  P  K  P  G  S  P  V  S  A  S  P  T

781   TAACTCCTACCCTATTTAAACAAGAACGCGACGAAATCCCTCTTGAACGGATTCCTTTCC
      L  T  P  T  L  F  K  Q  E  R  D  E  I  P  L  E  R  I  P  F

841   CCACACCCTCTATCACCGACTACTCCCCTACCTTGAGGCCTTCCACTCTGGCTGAGTCCT
      P  T  P  S  I  T  D  Y  S  P  T  L  R  P  S  T  L  A  E  S

901   CCGACGTGACACAACATCCTGCAGcggtgttgtgcgacctgcagTGTCCGTCGCTGGACT
      S  D  V  T  Q  H  P  A                        V  S  V  A  G  L

961   CGAAGGAGAAGGAAGTGCCCTCTCTCTCTTTGACGTCGGCTCAAACCCTGAACCTCACGC
        E  G  E  G  S  A  L  S  L  F  D  V  G  S  N  P  E  P  H  A
```

FIG._28B

```
1021  TGCCGATGATCTTGCAGCTCCTCTTTCTGACGATGACTTCCACCGCCTATTCAACGTTGA
        A  D  D  L  A  A  P  L  S  D  D  D  F  H  R  L  F  N  V  D

1081  TTCACCCGTTGGGTCAGATTCTTCAGTCCTTGAAGACGGGTTCGCCTTTGACGTTCTCGA
        S  P  V  G  S  D  S  S  V  L  E  D  G  F  A  F  D  V  L  D

1141  CGGAGGAGATCTATCAGCATTTCCATTTGATTCTATGGTTGATTTCGACCCCGAATCTGT
        G  G  D  L  S  A  F  P  F  D  S  M  V  D  F  D  P  E  S  V

1201  TGGCTTCGAAGGCATCGAGCCGCCCCACGGTCTTCCGGATGAGACTTCTCGCCAGACTTC
        G  F  E  G  I  E  P  P  H  G  L  P  D  E  T  S  R  Q  T  S

1261  TAGCGTGCAACCCAGCCTTGGCGCGTCCACTTCGCGATGCGACGGGCAGGGCATTGCAGC
        S  V  Q  P  S  L  G  A  S  T  S  R  C  D  G  Q  G  I  A  A

1321  TGGCTGTTAGCGAGCAGTTTCGCCAGGGAGATGCATCGGCTGTCGATGGTAACGGAGTCC
        G  C
1381  AATGGAGCTGGGAGTCTTTGTTGACCTTGGCGTGGACGATAGACCTACTCGAACAGCCGG
1441  GACGACGCAAACGAATCTTGAGCGGTTTGAAATCAGCGAAAACTGGACGGCGAAGTAATA
1501  TTGGCAAGTCTCAAAGGAGTACACGGAGTTCATGGAGTTCACGAAGCACCCAAGAGGCGT
1561  TGACGTCTCTCCTTATGGGCAAGCATAGTTGAGGTTCCGGCTGTAAATTATCATAAATCC
1621  TTATAATTTTATTCTAGATTTCAATACAGCAGTTGATTGTCTGCTCATC
```

FIG._28C

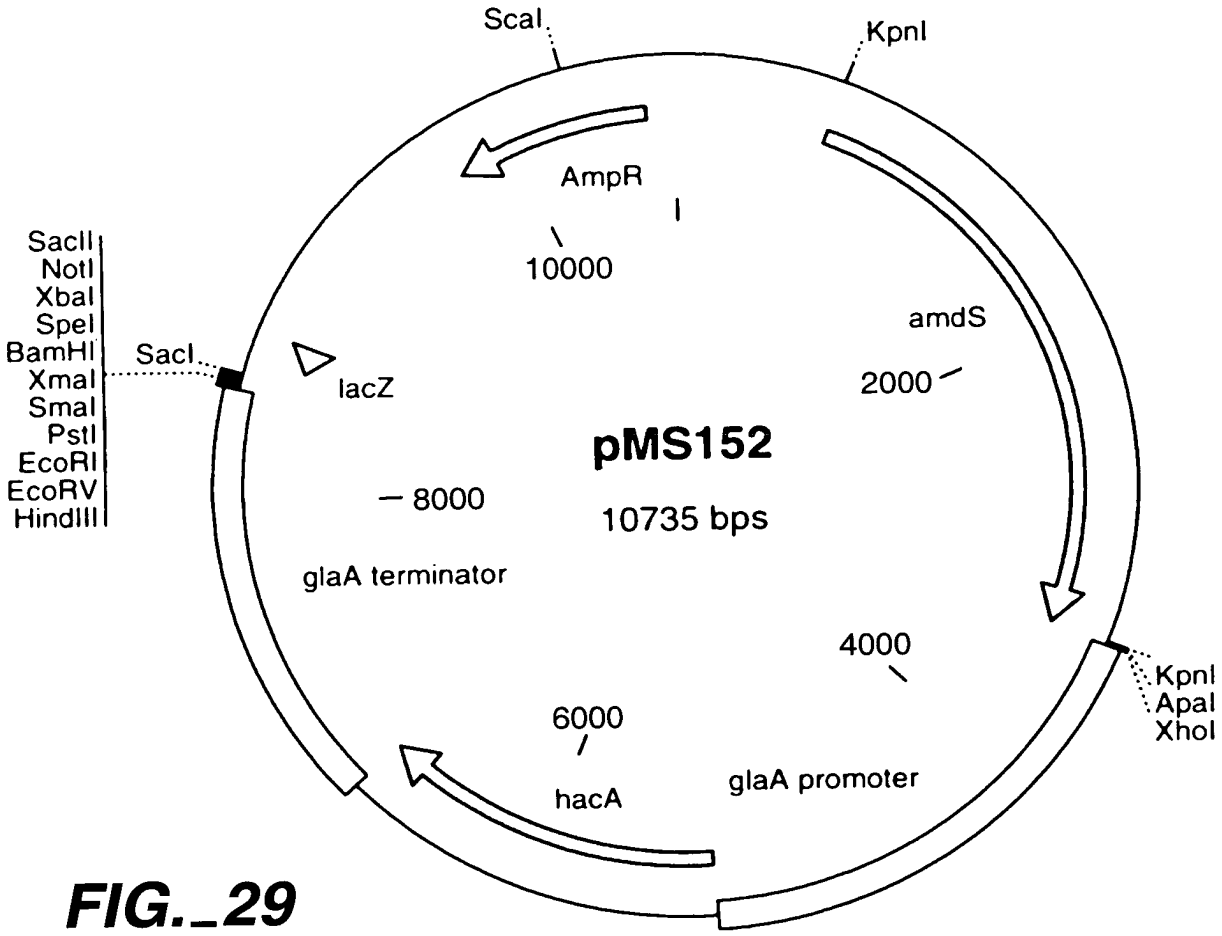

ScaI
KpnI
AmpR
10000
amdS
2000
SacII
NotI
XbaI
SpeI
BamHI
XmaI
SmaI
PstI
EcoRI
EcoRV
HindIII
SacI
lacZ
pMS152
10735 bps
8000
glaA terminator
4000
KpnI
ApaI
XhoI
6000
hacA
glaA promoter

FIG._29

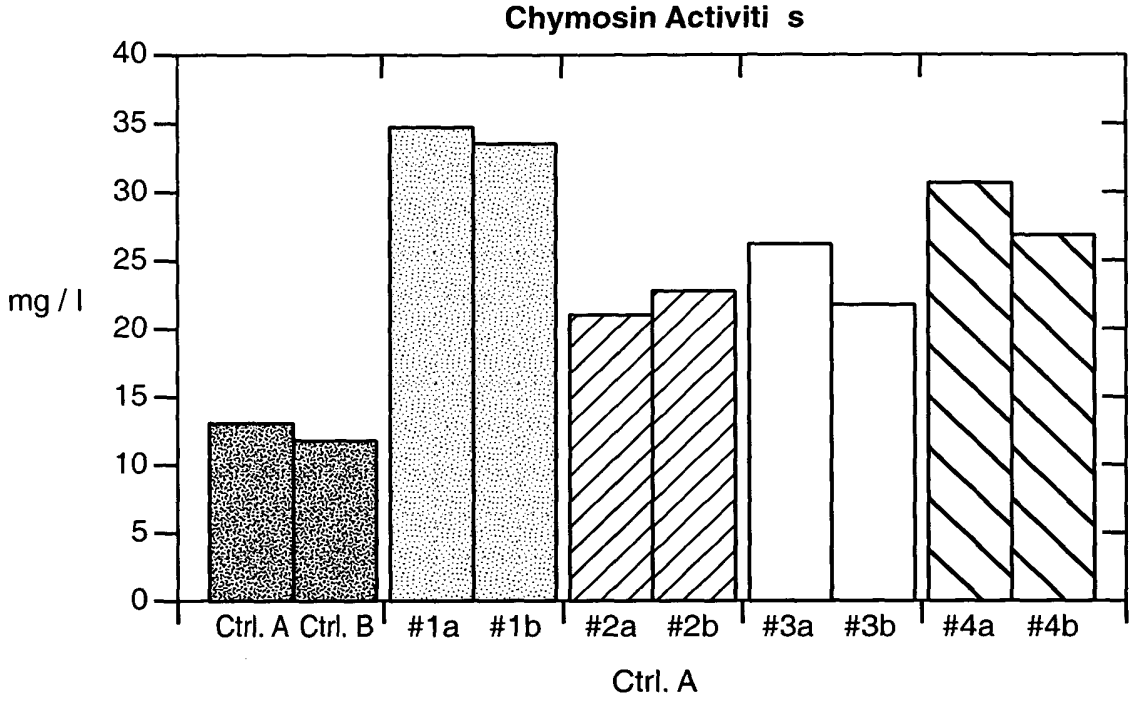
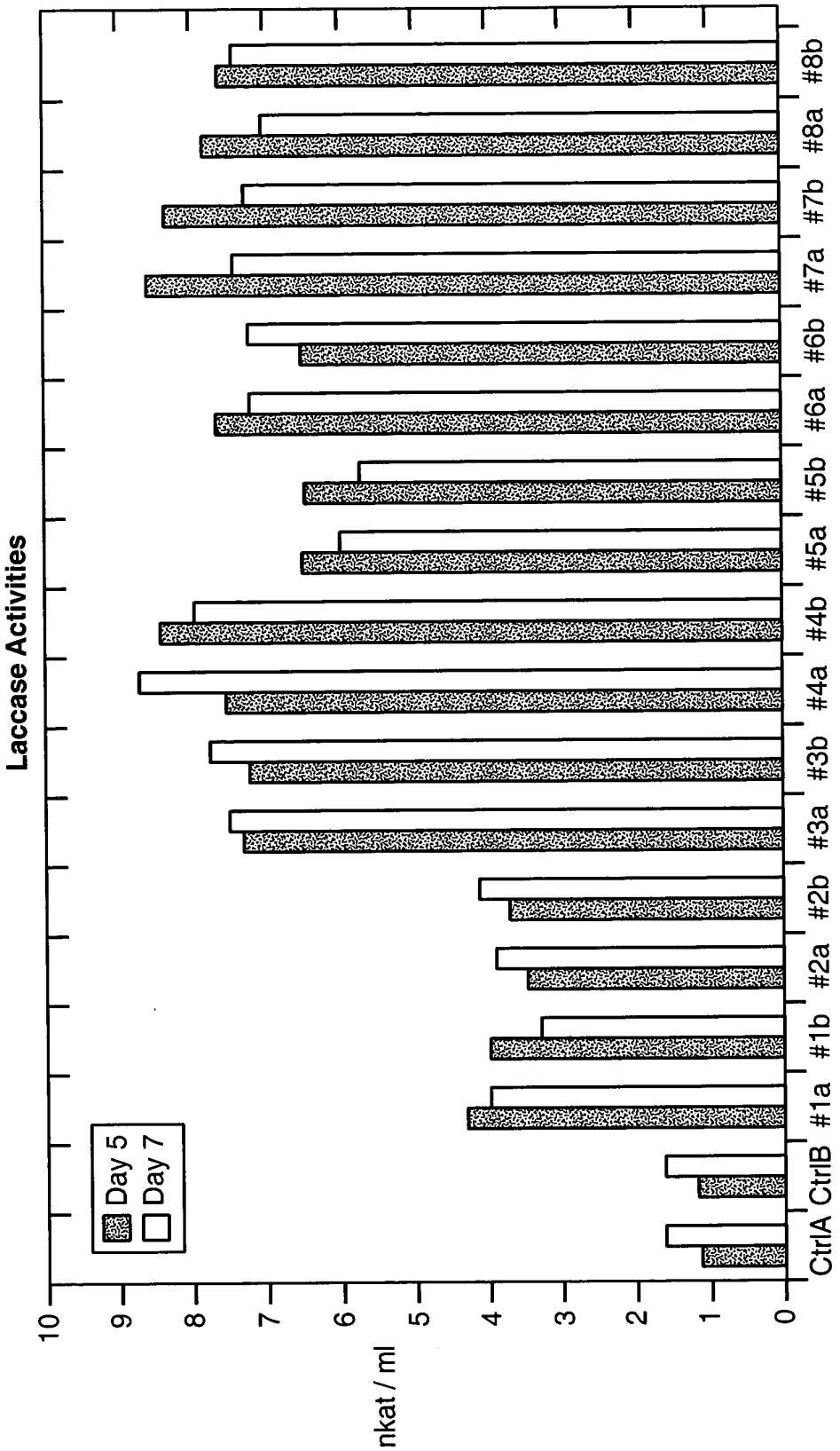

Chymosin Activiti s
mg / l
40
35
30
25
20
15
10
5
0
Ctrl. A
Ctrl. B
#1a
#1b
#2a
#2b
#3a
#3b
#4a
#4b
Ctrl. A

FIG._30


Laccase Activities
nkat / ml
0
1
2
3
4
5
6
7
8
9
10
Day 5
Day 7
CtrlA
CtrlB
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#1b
#2a
#2b
#3a
#3b
#4a
#4b
#5a
#5b
#6a
#6b
#7a
#7b
#8a
#8b

FIG._31